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Roche

Diagnostics

Tools for Amplification

Programmed for performance. Fast. Effective. Easy.

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Roche Applied Science is strongly committed to continue serving the scientific community with new innovative systems and reagents – driven by our philosophy that innovation and quality are the driving factors for success. We are able to help researchers by providing total solutions in the form of cutting edge systems and fully integrated reagents. This guide is designed to assist you in selecting the optimum product based on the criteria that are most important to you.

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Template Preparation | RT-PCR, PCR, Nucleotides | Detection & Processing

Benefits

You can have it all

Accelerate amplification results
Use tailor-made products with the added advantage of an Integrated Solutions concept that can bring out the best in your research. The success of your application depends on every step performed.

Obtain consistent results
Benefit from Roche Applied Science quality standards including ISO 9001:2000 certification to produce consistent results – tube-to-tube, experiment-to-experiment – with products that exemplify lot-to-lot consistency.

Rely on best performing hot start technology
Achieve higher specificity, sensitivity and yield, and enjoy comfortable reaction setup at room temperature. All FastStart products are based on Roche's proprietary hot start technology of chemical modification of proteins.

Use Roche as the information provider
Benefit from our committed Technical Service teams that offer customized services worldwide. A large variety of application manuals, internet tools, Biochemica newsletter, and product documentation are also excellent resources for your research needs.

Gain from the "Gold Standards"
Rely on our products and services, and stay in a constant contact with us to further develop new ideas into products. Roche Applied Science will continue to set the long standing standards.

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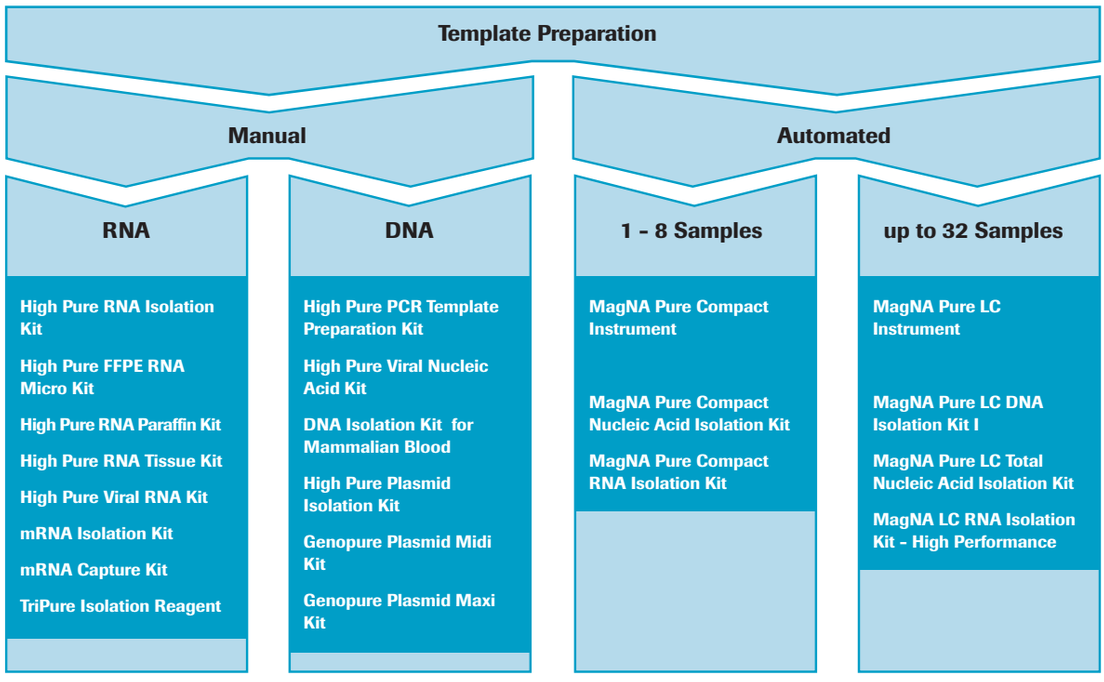
Template Preparation – Overview

Maximize your results using Roche Applied Science's purification and amplification products – designed to excel individually and perform well together.

Fundamental molecular biology techniques require high quality, highly purified nucleic acid samples prior to all further procedures. Choose from Roche Applied Science's broad range of easy-to-use, reliable products for the purification of genomic DNA, viral DNA, plasmid DNA, DNA fragments, as well as total, viral and messenger RNA.

- **Insist on unlimited flexibility.**
  DNA or RNA can be purified from numerous biological sources and at various scales.
- **Rely on tailor-made products.**
  Solutions are available for the most demanding applications.

- **Choose between manual or automated systems.**
  Optimal systems are available to meet your research needs.



For more information on manual nucleic acid purification, visit our special interest site at:
www.roche-applied-science.com/napure

For a complete overview of the wide variety of kits and reagents for automated nucleic acid purification, visit our special interest site at:
www.magnapure.com

Isolation of RNA

Recover high-quality RNA

Unpurified RNA templates are a common cause of RT-PCR failure. The following tables can help you find the best purification kit or reagent for your sample type and desired nucleic acid.

- **Save time.**
  High quality RNA can be isolated in a short time period.
- **Receive intact RNA.**
  Optimal RNA quality is a prerequisite for excellent results in all downstream applications.
- **Avoid RNA loss.**
  Precipitation or solvent extraction steps are not required.

Target Nucleic Acid	Origin/Source	Products
Total RNA	Mammalian cultured cells	High Pure RNA Isolation Kit TriPure Isolation Reagent
	Mammalian whole blood	
	Yeast cells	
	Gram positive and negative bacteria	
	Formalin-fixed, paraffin-embedded (FFPE) tissue sections	High Pure FFPE RNA Micro Kit High Pure RNA Paraffin Kit
	Solid tissues	High Pure RNA Tissue Kit

Target Nucleic Acid	Origin/Source	Products
Messenger RNA	Cultured cells	mRNA Isolation Kit mRNA Capture Kit
	Tissues	
	Total RNA	

Target Nucleic Acid	Origin/Source	Products
Viral RNA	Serum	High Pure Viral RNA Kit
	Plasma	
	Other body fluids (*e.g.*, tears, urine, breast milk)	
	Supernatant from cell cultures	

Product	Cat. No.	Pack Size
High Pure RNA Isolation Kit *	11 828 665 001	Up to 50 isolations
High Pure RNA Paraffin Kit *	03 270 289 001	Up to 100 isolations
High Pure RNA Tissue Kit *	12 033 674 001	Up to 50 isolations
mRNA Isolation Kit *	11 741 985 001	1 kit

Product	Cat. No.	Pack Size
mRNA Capture Kit *	11 787 896 001	192 isolations
High Pure Viral RNA Kit *	11 858 882 001	Up to 100 isolations
High Pure FFPE RNA Micro Kit *	04 823 125 001	Up to 50 isolations
TriPure Isolation Reagent	11 667 157 001 11 667 165 001	50 ml 200 ml

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Isolation of RNA

Recover high-quality RNA

High Pure FFPE RNA Micro Kit

Choose the High Pure FFPE RNA Micro Kit to isolate total RNA from 1 to 10 µm sections of formalin-fixed, paraffin-embedded (FFPE) tissue samples (*e.g.*, colon, breast, liver, kidney, spleen of mammalian species including human research samples) for direct use in RT-PCR. The isolated RNA is suitable for relative quantification of mRNA in RT-PCR using the LightCycler® Instruments or other real-time PCR systems.

- **Save time.**
  The kit protocol is fast and simple.
- **Obtain high yields.**
  Highly concentrated (10 µl) eluates with high recovery (≥ 80%) are obtained.
- **Insist on high sensitivity.**
  Small RNA fragments are also isolated from FFPE samples.

Product	Cat. No.	Pack Size
High Pure FFPE RNA Micro Kit *	04 823 125 001	Up to 50 isolations

High Pure RNA Isolation Kit

Choose the High Pure RNA Isolation Kit for rapid isolation of intact total RNA from mammalian cultured cells, blood, WBCs, yeast, gram positive bacteria and gram negative bacteria.

- **Process a wide variety of sample material.**
  A single sample preparation is completed in 25 minutes, and multiple in 45 minutes.
- **Prevent RNA loss.**
  Precipitation or solvent extraction steps are not required.
- **Avoid DNA contamination.**
  Integrated DNA digestion and DNase removal eliminate signals from genomic DNA.
- **Obtain concentrated RNA.**
  RNA is eluted in a 50 µl volume.

The RNA obtained is ideal for all subsequent molecular biology techniques and especially those that require the absence of genomic DNA. The intact, total RNA is ready-to-use in techniques, like cDNA library construction, RT-PCR, northern blotting, differential display, nuclease protection assay, primer extension, RACE and *in vitro* translation.

Product	Cat. No.	Pack Size
High Pure RNA Isolation Kit *	11 828 665 001	Up to 50 reactions

mRNA Capture Kit

Choose the mRNA Capture Kit for capturing of polyadenylated RNA from total RNA, cell lysates and tissue homogenates in 200 µl PCR tubes without prior RNA preparation. The method relies on the base-pairing between the Poly (A+) residues at the 3'p-end of the mRNA and kit's biotin-labeled oligo (dT) probe. The hybrid is then immobilized in the strepavidin-coated PCR tubes, and unbound contaminants removed by wash steps. The immobilized biotin-labeled oligo (dT)/poly (A+) hybrid can serve as a primer for the reverse transcriptase step in RT-PCR in the same tube.

- **Maximize convenience.**
  Combine rapid, efficient isolation of poly (A+) RNA with RT-PCR in a single tube to prevent sample loss, reduce handling time, and minimize the risk of contamination
- **Save time.**
  RNA is isolated with an innovative process that simplifies the handling of large number of samples.
- **Avoid background.**
  The kit produces excellent RT-PCR templates even from small amounts of starting material, or material containing low-abundance mRNAs.

Product	Cat. No.	Pack Size
mRNA Capture Kit *	11 787 896 001	192 isolations

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Isolation of DNA

Flexibility unlimited

Many of Roche Applied Science's products for DNA purification have a wide range of applications whereas others have been optimized for a special purpose.

- **Insist on unlimited flexibility.** High-quality DNA is obtained from most sources.
- **Improve PCR reproducibility and reliability.** PCR inhibitors are efficiently removed.

The following tables can help you find the best purification kit or reagent for your sample type and desired nucleic acid.

- **Allow long template applications.** High molecular weight DNA (30 – 50 kb) can be obtained.
- **Save time.** Multiple PCR templates are purified in minutes.

Target Nucleic Acid	Origin/Source	Products
Genomic DNA	Human whole blood	High Pure PCR Template Preparation Kit
	Buffy coat	
	Cultured mammalian cells	
	Mouse tails	
	Tissues	
	Yeast cells	
	Gram positive and negative bacteria	
	Formalin-fixed, paraffin-embedded tissue	
	Mammalian whole blood (up to 10 ml) Lymphocyte / Buffy coat	DNA Isolation Kit for Mammalian Blood

Target Nucleic Acid	Origin/Source	Scale	Products
Plasmid DNA	*E. coli* cultures	Small	High Pure Plasmid Isolation Kit
		Medium	Genopure Plasmid Midi Kit
		Large	Genopure Plasmid Maxi Kit

Target Nucleic Acid	Origin/Source	Products
Viral DNA	Serum	High Pure Viral Nucleic Acid Kit
	Plasma	
	Human whole blood	
	Supernatant from cell cultures	

Product	Cat. No.	Pack Size
High Pure PCR Template Preparation Kit *	11 796 828 001	Up to 100 isolations
DNA Isolation Kit for Mammalian Blood *	11 667 327 001	25 purifications
High Pure Plasmid Isolation Kit	11 754 777 001 11 754 785 001	50 purifications 250 purifications

Product	Cat. No.	Pack Size
Genopure Plasmid Midi Kit *	03 143 414 001	Up to 20 preparations
Genopure Plasmid Maxi Kit *	03 143 422 001	Up to 10 preparations
High Pure Viral Nucleic Acid Kit *	11 858 874 001	Up to 100 isolations

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Isolation of DNA

Flexibility unlimited

High Pure PCR Template Preparation Kit

Use the versatile High Pure PCR Template Preparation Kit to isolate genomic DNA from whole blood, buffy coat, cultured cells, tissue, yeast, bacteria, mouse tail, and other sample materials. Multiple PCR templates can be obtained in minutes with this fast and efficient kit. The single kit facilitates reproducibility and reliability for a multitude of applications, including routine or long PCR, microsatellite analysis, Southern blotting, qPCR, microarray analysis, and restriction digest.

- **Insist on unlimited flexibility.** This versatile kit works well with a wide variety of sample materials.
- **Enhance amplification results.** PCR inhibitors are efficiently removed.
- **Facilitate long template applications.** High molecular weight DNA can be isolated.
- **Be confident in your PCR results.** High-quality DNA obtained using this kit delivers reproducible results, even in real-time PCR.

*For the amplification of fragments between 20 – 35 kb the Expand 20 kb^{PLUS} PCR System is the recommended PCR reagent (see page 24).

Product	Cat. No.	Pack Size
High Pure PCR Template Preparation Kit *	11 796 828 001	Up to 100 purifications

DNA Isolation Kit for Mammalian Blood

Choose the DNA Isolation Kit for Mammalian Blood for the isolation of genomic DNA from mammalian whole blood, lymphocyte and buffy coat. The kit provides all necessary reagents for the rapid isolation of purified genomic DNA, free of contaminating heme and proteins. The resulting DNA is pure, has a high molecular weight, and is ready for use in all applications, including genomic Southern blots, and standard and long template PCR.

- **Process multiple samples simultaneously.** DNA is easily purified in less than 90 minutes.
- **Use cost-effective kits.** The convenient kit purifies DNA for the cost of most homebrew methods.
- **Enjoy consistent and reliable results.** The kit permits analysis of different sample volumes (1 to 10 ml) with varying amounts of leukocytes.

Product	Cat. No.	Pack Size
DNA Isolation Kit for Mammalian Blood *	11 667 327 001	25 isolations from 10 ml blood

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Enzymes to Degrade Cellular Components

Rely on the 50-year tradition in high-quality enzymes

Proteinase K recombinant, PCR-Grade

The enzyme is the recombinant form of the enzyme from *Tritirachium album* expressed in *Pichia pastoris*. The enzyme is extremely effective on native proteins and can therefore be used to rapidly inactivate endogenous nucleases during nucleic acid isolation for cloning, sequencing, PCR, and RT-PCR. The absence of nucleic acid in reagents which are used in PCR and RT-PCR assays is imperative. No exogenous nucleic acid should be introduced during the isolation step.
The additional low bioburden content guarantees improved product stability and security.

- **Choose a unique tool.**
  This is the only available recombinant Proteinase K on the market with unmatched quality.
- **Insist on contaminant-free enzyme.**
  Reliable and sensitive quality control tests ensure detection of trace contaminants.
- **Work with a versatile tool.**
  The contaminating activities are easily removed to obtain highly pure RNA and DNA from tissues or cell lines.
- **Analyze membrane structures.**
  The protease modifies proteins and glycoproteins on cell surfaces.

Product	Cat. No.	Pack Size
Proteinase K recombinant PCR-Grade (lyophilizate) *	03 115 836 001	25 mg
	03 115 879 001	100 mg
	03 115 801 001	2 x 250 mg
	03 115 852 001	4 x 250 mg
Proteinase K recombinant, PCR-Grade (solution) *	03 115 887 001	1.25 ml
	03 115 828 001	5 ml
	03 115 844 001	25 ml

DNase I recombinant, RNase-free

Easily remove even trace quantities of DNA from molecular biology reactions by using our recombinant DNase I. DNase I recombinant treatment results in high quality, intact, undegraded RNA ready for further downstream applications like RT-PCR, degradation of genomic DNA template in *in vitro* transcription reactions, foot-printing analysis of DNA-protein-interaction, and microarray target control. The highly purified enzyme is produced in *Pichia pastoris*, in a recombinant form of bovine DNase I.

- **Eliminate DNA contamination.**
  DNA is efficiently removed from any RNA sample.
- **Benefit from an animal-free product.**
  The entire production process and product are animal free.
- **Ensure maximal activity.**
  The enzyme is supplied with an optimized incubation buffer.
- **Reduce contamination risk.**
  There is no detectable RNase or protease activity in the enzyme preparation.

Product	Cat. No.	Pack Size
DNase I recombinant, RNase-free	04 716 728 001	10, 000 units

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RNA Stabilization

Maximize the protection of your precious RNA

Protector RNase Inhibitor

Inhibit a wide spectrum of RNases (Table 1) with Protector RNase Inhibitor and protect your RNA samples from RNases, and thereby from degradation. This thermostable enzyme is fully active during cDNA synthesis, (*e.g.*, thermostable reverse transcriptases like Transcriptor Reverse Transcriptase). The versatile Protector RNase Inhibitor can be used in any application where RNases are a potential problem.

- **Generate full-length transcripts.** Protector RNase Inhibitor is active at pH 5.0 – 9.0 and at temperatures between 25°C to 55°C (partial activity is still measurable at 60°C). It works at elevated temperatures, thereby overcoming secondary structure in RNA and facilitating optimal reaction conditions.
- **Eliminate any interference.** Even at high concentrations, Protector RNase Inhibitor does not interfere with other enzymes and systems commonly used to analyze RNA.
- **Insist on a highly-purified preparation.** Each batch is function-tested using techniques like quantitative RT-PCR to ensure the absence of endonucleases, ribonucleases, or nicking activities.

Type of RNase	Amount Inhibited
RNase A	Up to 1 ng
RNase B	Up to 160 pg
RNase T2	Up to 0.03 U/µl reaction volume

Table 1: Inhibition of different types of RNases by Protector RNase Inhibitor (20 units / 20 µl reaction).

Application	Products Tested
RT-PCR	Transcriptor Reverse Transcriptase in combination with 1. Taq DNA Polymerase 2. FastStart Taq DNA Polymerase AMV Reverse Transcriptase in combination with Expand High Fidelity PCR System (as in the Titan One Tube RT-PCR System)
cDNA synthesis	cDNA Synthesis System Microarray cDNA Synthesis Kit*
qRCR	LightCycler® Instrument and related kits
in vitro transcription/ translation	T7 RNA Polymerase in wheat germ lysate
	* included in kit's enzyme mix

Table 2: Protector RNase Inhibitor applications
Result: Protector RNase Inhibitor is compatible with a wide range of products and applications.

MS2 RNA – 2.5 µg in a reaction volume of 20 µl

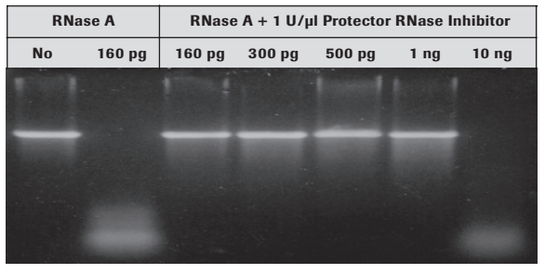

Figure 1: Protection of RNA from degradation by RNase A with Protector RNase Inhibitor. MS2 RNA (2.5 µg) was incubated in a reaction volume of 20 µl in the absence or presence of various amounts of RNase A.
Result: Subsequent gel electrophoresis revealed that Protector RNase Inhibitor inhibited RNase A at levels as high as 1 ng/20 µl.

Product	Cat. No.	Pack Size
Protector RNase Inhibitor *	03 335 399 001 03 335 402 001	2,000 units 10,000 untis (5 x 2,000 units)

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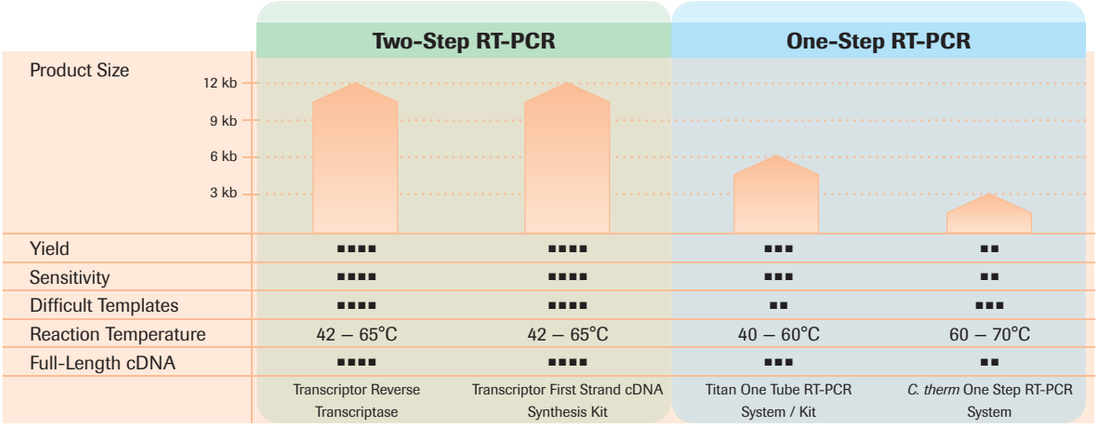
RT-PCR – Overview

Perform a broad array of applications, and find out particular strengths that can be leveraged in your specific applications with Roche Applied Science's extensive product line for amplification.

Choose the right reverse transcriptase for your RT-PCR application. The overall performance depends on the choice between one-step or two-step RT-PCR, and the selection of reverse transcriptase.

Success in RT-PCR and cDNA synthesis requires the effective purification and protection of RNA template. For more information see page 5.

- **Achieve unsurpassed sensitivity.** Exceptionally small amounts of template are sufficient for one- or two-step RT-PCR.
- **Obtain full-length cDNA transcripts.** mRNA up to 12 kb can be reverse transcribed.
- **Overcome difficult templates.** High secondary structure RNA is not a problem.
- **Efficiently label cDNA.** The amplification product can be used in various applications.



Yield	■■■■	■■■■	■■■	■■
Sensitivity	■■■■	■■■■	■■■	■■
Difficult Templates	■■■■	■■■■	■■	■■■
Reaction Temperature	42 – 65°C	42 – 65°C	40 – 60°C	60 – 70°C
Full-Length cDNA	■■■■	■■■■	■■■	■■
	Transcriptor Reverse Transcriptase	Transcriptor First Strand cDNA Synthesis Kit	Titan One Tube RT-PCR System / Kit	*C. therm* One Step RT-PCR System

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Two-Step RT-PCR

Increase the power and sensitivity of cDNA synthesis

Transcriptor Reverse Transcriptase

Choose the new recombinant Transcriptor Reverse Transcriptase expressed in *E. coli*., for all your RNA applications involving mRNA, total RNA, viral RNA, or *in vitro*-transcribed RNA from a variety of sources. The enzyme has RNA-directed DNA polymerase activity, DNA-dependent DNA polymerase activity, unwinding activity, and RNase H activity that degrades RNA in RNA:DNA hybrids. The latter circumvents the need to perform an additional time-consuming RNase H incubation step after reverse transcription. This shortens the reaction time and reduces costs.

- **Achieve high sensitivity in two-step RT-PCR.**
  Transcriptor Reverse Transcriptase is used in conventional thermal cyclers and real-time PCR instruments (*e.g.*, the LightCycler® Instruments).
- **Obtain more full-length transcripts - up to 12 kb.**
  cDNA libraries with large inserts can be generated.
- **Reverse transcribe difficult templates.**
  It works well at elevated temperatures thereby overcoming RNA secondary structure (*e.g.*, GC-rich RNA templates) facilitating optimal reaction conditions.
- **Efficiently label cDNA.**
  Cy3-, Cy5-, DIG-, biotin-, or aminoallyl-labeled nucleotides are incorporated during cDNA synthesis.

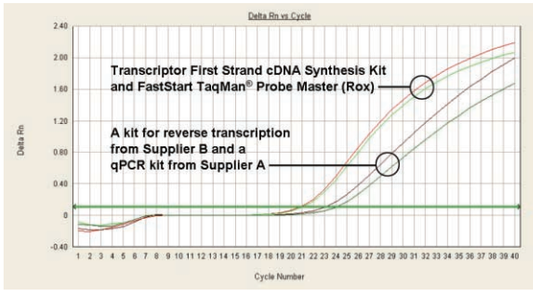

Figure 2: Sensitive and efficient real-time PCR with Transcriptor Reverse Transcriptase.
Beta-2-microglobulin (β2M) mRNA was reverse transcribed and amplified using Transcriptor Reverse Transcriptase (supplied in Transcriptor First Strand cDNA Synthesis Kit) in combination with Roche's FastStart TaqMan® Probe Master. For comparison, the same assay was performed using supplier B's Reverse Transcriptase in combination with a qPCR kit from supplier A.
Result: The reactions with Transcriptor Reverse Transcriptase and the FastStart TaqMan® Probe Master produced the best sensitivity.

Product	Cat. No.	Pack Size
Transcriptor Reverse Transcriptase *	03 531 317 001	250 units (25 reactions)
	03 531 295 001	500 units (50 reactions)
	03 531 287 001	4 x 500 units (200 reactions)

Product Size	3 kb 6 kb 9 kb 12 kb
Reaction Temperature	40 °C 50 °C 60 °C 70 °C 80 °C
Yield/Sensitivity	▪▪▪▪/▪▪▪▪
Difficult Templates/ Full-Length cDNA	▪▪▪▪/▪▪▪▪

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Transcriptor First Strand cDNA Synthesis Kit

Use the Transcriptor First Strand cDNA Synthesis Kit to reverse transcribe RNA into single-stranded cDNA. The resulting cDNA can be used directly for subsequent PCR amplification with gene-specific primers in conventional thermal cyclers and real-time PCR instruments (*e.g.*, the LightCycler® Instruments), or in other downstream applications. The kit conveniently provides all components for cDNA synthesis, including primers, nucleotides and controls for use with both conventional thermal cyclers and real-time PCR instruments. There are no additives in the RT buffer system that interfere with – or inhibit – the subsequent PCR reaction, yielding reliable and sensitive results.

- **Enjoy all the benefits of Transcriptor Reverse Transcriptase.**
  Transcriptor Reverse transcriptase is the core component of this kit.
- **Use the kit's unique anchored-oligo $(dT)_{18}$ primer.**
  The anchored-oligo $(dT)_{18}$ primer binds at the beginning of the poly(A) tail, rather than mis-priming at an internal site within the tail.
- **Easily monitor reverse transcription.**
  For control reactions the ready-to-use solutions of RNA and PBGD primers are supplied.
- **Save your RNA from degradation.**
  Protector RNase Inhibitor is included in this kit.
- **Rely on strict quality control.**
  The kit is function-tested for PCR on conventional thermal cyclers and on the LightCycler® Instruments.

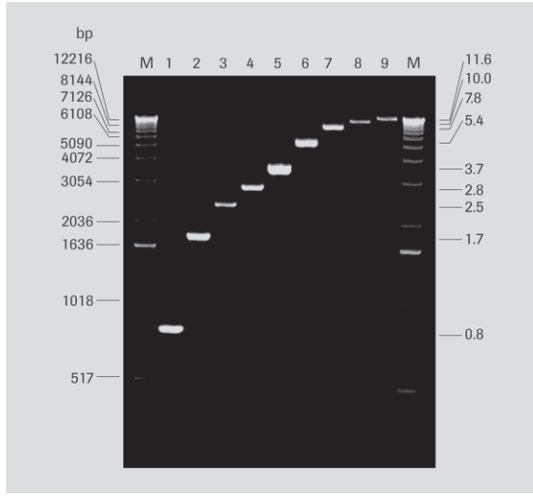
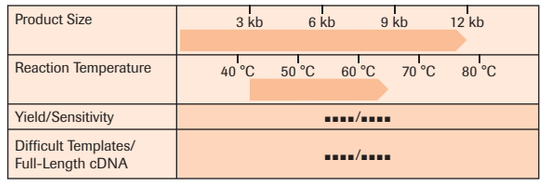


Figure 3: Produce full-length cDNA with the Transcriptor First Strand cDNA Synthesis Kit.
Reverse transcription was performed on 2 µg total RNA (all lanes except lane 3) from human skeletal muscle, using the kit's anchored oligo $(dT)_{18}$ primer. In a subsequent PCR, various fragments of the dystrophin gene were amplified using either the Expand High Fidelity (Lanes 1, 2, 4) or the Expand Long Template (5 to 9) PCR Systems. In lane 3, 1 µg of total RNA from human liver cells was used as template for the reverse transcription, and Expand High Fidelity was used to amplify an ApoB gene fragment in the subsequent PCR.

Product	Cat. No.	Pack Size
Transcriptor First Strand cDNA Synthesis Kit *	04 379 012 001	1 kit (50 reactions)



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One-Step RT-PCR

Unsurpassed sensitivity

Titan One Tube RT-PCR System

Choose the Titan One Tube RT-PCR System for unmatched sensitivity and convenience, avoiding contamination through one-step RT-PCR. This highly sensitive technique enables determining the presence or absence of RNA templates, or to quantify the levels of gene expression through RNA analysis. Combining three distinct enzymes, the Titan System exploits the high thermal stability of AMV Reverse Transcriptase for the cDNA synthesis step, reducing secondary structure by permitting reverse transcription at 42°C to 60°C. Then the cDNA is amplified by PCR using the optimized enzyme blend of Expand High Fidelity PCR System. RT-PCR products can be generated up to 6 kb.

- **Save precious starting material.**
  One-step RT-PCR is a highly sensitive technique and avoids false negatives.
- **Improve specificity.**
  The reaction temperatures can be increased up to 60°C, reducing RNA secondary structure, resulting in a larger number of full-length transcripts.
- **Obtain higher yields and a three-fold increase in fidelity.**
  The Titan System exploits the high yield and enhanced fidelity of the Expand High Fidelity PCR System enzyme blend for the PCR step, ensuring higher RT-PCR sensitivity.
- **Choose either a complete kit or a reagent system.**
  The Titan One Tube RT-PCR Kit conveniently combines the enzymes from the Titan One Tube System with nucleotides, RNase Inhibitor and control reactions.

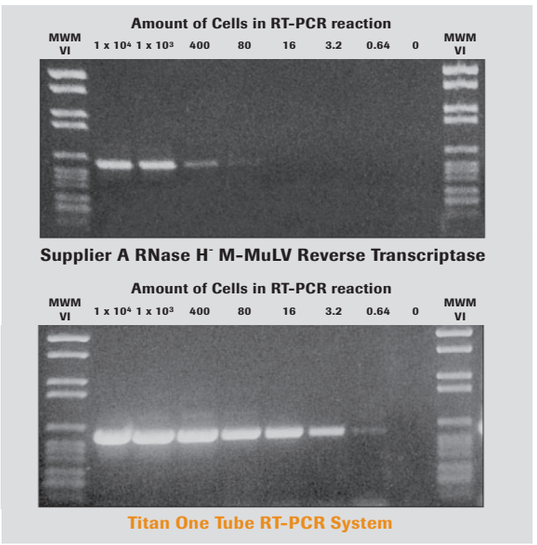
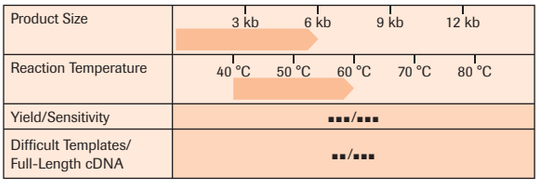


Figure 4: One-step RT-PCR of β-actin transcript from K-562 cells.
mRNA from serially diluted cell lysates was captured in streptavidin-coated PCR tubes using the mRNA Capture Kit (see page 6). RT-PCR was performed with the Titan One Tube RT-PCR System (RT at 50°C) or with RNase H⁻ M-MuLV Reverse Transcriptase from Supplier A (RT at 42°C) and Taq DNA polymerase, according to each supplier's protocol.
Result: The Titan One Tube RT-PCR System delivers more than 100-times greater sensitivity than the two-step method with Supplier A's reverse transcriptase.

Product	Cat. No.	Pack Size
Titan One Tube RT-PCR Kit +	11 939 823 001	50 reactions (including 10 control reactions)
Titan One Tube RT-PCR System +	11 888 382 001 11 855 476 001	25 reactions 100 reactions



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One-Step RT-PCR

Overcome difficult RNA templates

C. therm. Polymerase One-Step RT-PCR System

Easily overcome secondary structure and transcription problems encountered during one-step RT-PCR by choosing *C. therm.* Polymerase (Klenow fragment of DNA polymerase from *Carboxydothermus hydrogenoformans*). This magnesium-dependent polymerase exhibits optimal activity when reverse transcribing between 60°C and 70°C (maximum of 72°C), dramatically reducing RNA secondary structure and increasing transcription efficiency for RT-PCR products up to 3 kb. For the amplification step this system relies on the robust Taq DNA Polymerase.

- **Amplify difficult templates.**
  The option of DMSO titration and reverse transcription at temperatures up to 70°C allow the amplification of complicated sequences like GC-rich RNA.
- **Be confident in your RT-PCR results.**
  Consistent results can be obtained even in the presence of high amounts of nonspecific RNA.
- **Avoid false negatives.**
  The special enzyme mix allows the incorporation of dUTP and reduces the risk of carryover contamination in combination with Uracil-DNA Glycosylase.

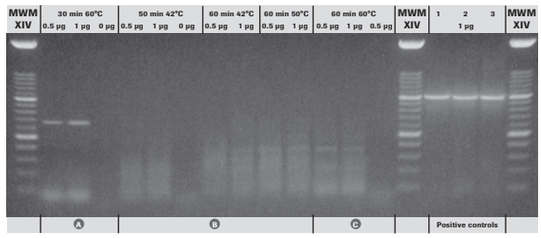
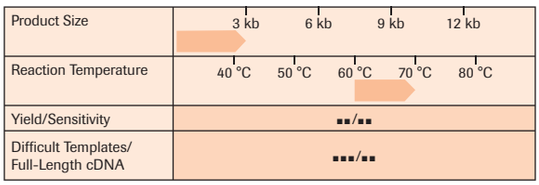


Figure 5: One-step RT-PCR of a 650 bp TGFβ mRNA. Total RNA isolated from the K-562 cell line was used as a template for *C. therm.* Polymerase, Supplier B's RNase H⁻ M-MuLV Reverse Transcriptase, or Supplier C's AMV Reverse Transcriptase. Additives were used to dissolve RNA secondary structure. 997-bp β-actin fragment from mouse was used as a positive control (1 µg total cDNA/reaction). Taq DNA Polymerase was used for all amplification steps.
Panel A and control lane 1: *C. therm.* Polymerase for 30 minutes at 60°C
Panel B and control lane 2: Supplier B's RNase H⁻ M-MuLV RT for 50 minutes at 42°C
Panel C and control lane 3: Supplier C's AMV RT for 60 minutes at 42°C
Result: *C. therm.* works on templates (Panel A) that other systems fail to amplify (Panels B and C). Only *C. therm.* Polymerase produced a visible product.

Product	Cat. No.	Pack Size
C. therm. Polymerase One-Step RT-PCR System [+,‡]	12 016 338 001 12 016 346 001	50 reactions 250 reactions



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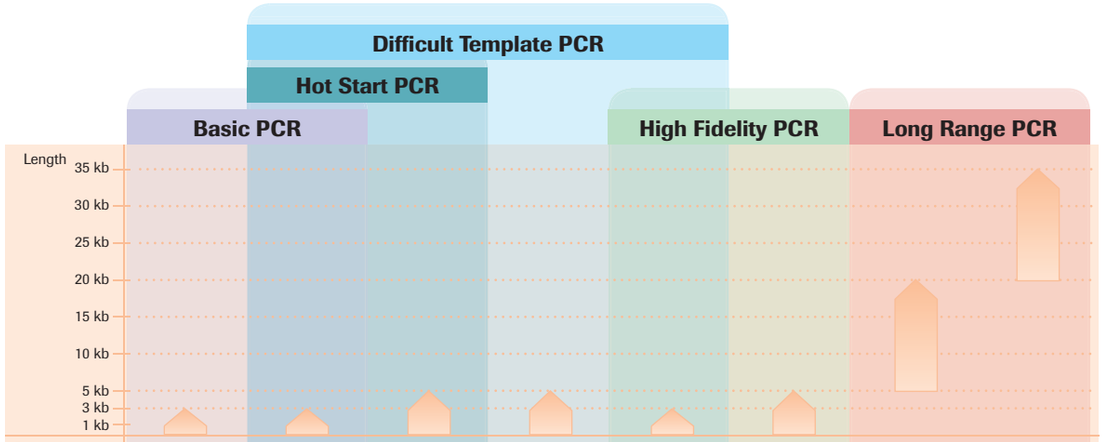
PCR – Overview

Roche Applied Science provides an extensive product line to meet all of your amplification needs. With more than 40 years of enzyme-purification expertise, Roche Applied Science has mastered the science of enzyme blending to bring you premium performance PCR and RT-PCR products.

- **Set your sights on the new standard.**
  Improved results are seen on upgrading to hot start PCR with FastStart Taq DNA Polymerase, and FastStart High Fidelity PCR System.
- **Insist on excellent fidelity.**
  Robust buffer systems facilitate outstanding yields and accuracy without optimization.
- **Conquer difficult assays.**
  Roche Applied Science's optimally designed systems enable the amplification of the most difficult templates, or multiple fragments.
- **Amplify longer fragments.**
  More full-length product – up to 35 kb – is obtained with higher fidelity and yields from complex genomic DNA.



	Taq DNA Polymerase	FastStart Taq DNA Polymerase	FastStart High Fidelity PCR System	GC-RICH PCR System	Pwo SuperYield DNA Polymerase	Expand High Fidelity^{PLUS} PCR System	Expand Long Template PCR System	Expand 20kb^{PLUS} PCR System
Specificity	▪▪	▪▪▪▪	▪▪▪▪	▪▪▪	▪▪	▪▪▪	▪▪▪	▪▪
Sensitivity	▪▪	▪▪▪▪	▪▪▪▪	▪▪▪	▪▪	▪▪▪	▪▪	▪▪
Robustness	▪▪	▪▪▪	▪▪▪	▪▪▪▪	▪▪	▪▪▪	▪▪▪	▪▪▪
Accurracy*		1 x	4 x	3 x	18 x	6 x	3 x	2 x
Carryover Prevention	yes	yes	yes	no	no	yes	no	no
Master Mix available	yes	yes	no	no	yes	yes	no	no

* compared to Taq DNA Polymerase

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Hot Start PCR

Set your sights on the new standard for everyday PCR

FastStart Taq DNA Polymerase

Use FastStart Taq DNA Polymerase for all basic PCR applications and overcome the poor performance of non-hot start polymerases. FastStart Taq DNA Polymerase is a thermostable, chemically modified form of recombinant Taq DNA Polymerase. It delivers superior results for fragments up to 3 kb, thanks to its unique enzyme design and optimized buffer system. It is inactive at temperatures below 75°C, but is activated by a two- to four-minute incubation step at 95°C.

- **Insist on higher specificity, sensitivity, and yield.**
  Hot start PCR dramatically improves the overall PCR performance.
- **Challenge even the most problematic DNA.**
  The revolutionary GC-RICH Solution, a PCR additive that reduces template secondary structure, is conveniently supplied with this product.
- **Conveniently use robotic pipetting systems.**
  The PCR setup can be performed at room temperature due to the enzyme's initial inactivity.
- **Make PCR setup easier.**
  Manual hot start, wax barriers, beads, or the need to set up PCRs on ice can be eliminated when using this hot start enzyme.
- **Prevent carryover contamination.**
  This enzyme allows the incorporation of modified nucleotides and dUTP. In combination with Uracil-DNA Glycosylase, it safeguards PCR from cross contamination.

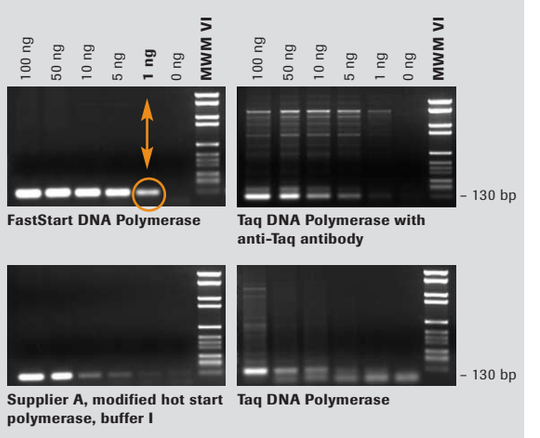
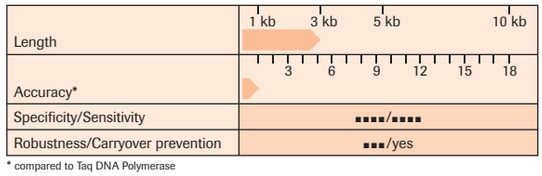


Figure 6: Amplification of a 130-bp fragment from the plasminogen activator (tPA) gene. Varying amounts of human genomic DNA were used for the amplification of a 130-bp fragment from the tissue plasminogen activator (tPA) gene.
Result: FastStart Taq DNA Polymerase produced the best specificity and sensitivity, even from the lowest amount of template.

Product	Cat. No.	Pack Size
FastStart Taq DNA Polymerase▼	12 158 264 001	50 units
	12 032 902 001	100 units
	12 032 929 001	2 x 250 units
	12 032 937 001	4 x 250 units
	12 032 945 001	10 x 250 units
	12 032 953 001	20 x 250 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.



* compared to Taq DNA Polymerase

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Hot Start PCR

Maximize convenience in high-performance hot start PCR

FastStart PCR Master

Make hot start PCR virtually effortless with the new FastStart PCR Master – a ready-to-use, double-concentrated hot start master mix. It contains FastStart Taq DNA Polymerase, our unmatched PCR-Grade Nucelotides, and all other reagents (except PCR primers and template) required for running every-day PCR and two-step RT-PCR on thermal cycler instruments.

- **Maximize convenience.**
  All you need to provide is primers, template, and water – the double-concentrated master mix contains everything else you need.
- **Simplify a variety of PCR applications.**
  The convenient master mix can be used to amplify fragments in routine, high-throughput PCR, or direct colony PCR.
- **Improve reliability, and reduce risk of contamination.**
  Fewer pipetting steps are necessary, thereby limiting sources of error and contamination.
- **Set up reactions with robotic pipetting stations.**
  The heat-activated polymerase-based mix is stable for 24 hours at room temperature.
- **Reduce setup time.**
  FastStart PCR Master can be stored at +2 to +8°C – ready for immediate use – for up to one month.

Amplify genomic and cDNA fragments up to 2 kb.

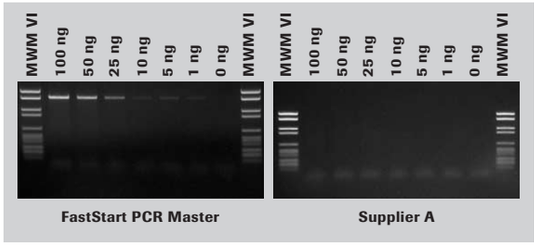
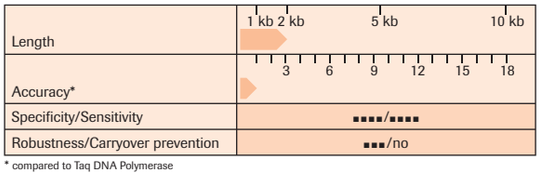


Figure 7: Amplification of a 1.8-kb fragment of the erythropoietin gene from different amounts of human genomic DNA using Roche Applied Science's FastStart PCR Master or a master mix from Supplier A.
Result: FastStart PCR Master amplified the fragment with high specificity, whereas Supplier A's master mix showed no amplification product.

Product	Cat. No.	Pack Size
FastStart PCR Master ▼	04 710 436 001	2 x 1.25 ml
	04 710 444 001	8 x 1.25 ml
	04 710 452 001	10 x 5 ml



* compared to Taq DNA Polymerase

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Hot Start PCR

Challenge the performance of your hot start PCR

FastStart High Fidelity PCR System

Combine all the features of FastStart Taq DNA Polymerase with four times the accuracy and the ability to amplify fragments up to 5 kb with the FastStart High Fidelity PCR System. It comprises a unique blend of FastStart Taq DNA Polymerase and a novel thermostable proofreading protein that carries no polymerase activity. Both proteins are chemically modified and inactive below 75°C, but are activated by heating to 95°C for 2 minutes.

- **Amplify longer templates.**
  The reagent allows the amplification of a variety of DNA and cDNA fragments up to 5 kb.
- **Increase fidelity.**
  The enzyme blend shows an 4-fold higher fidelity compared to Taq DNA Polymerase and FastStart Taq DNA Polymerase.
- **Achieve excellent performance in multiplex PCR.**
  The system amplifies multiple PCR fragments simultaneously. For difficult multiplex reactions the ultimate performance is obtained in combination with our PCR Optimization Kit.
- **Challenge even the most problematic DNA.**
  DMSO, a PCR additive that facilitates working with difficult templates, is conveniently supplied with this product.

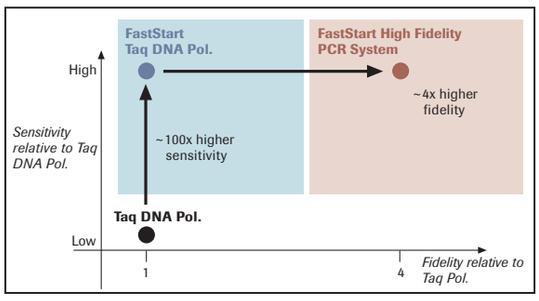


Figure 8: Improved features of FastStart High Fidelity PCR System.

Amplify genomic and cDNA fragments up to 5 kb.

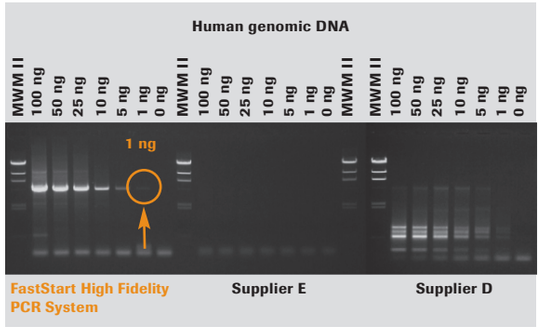
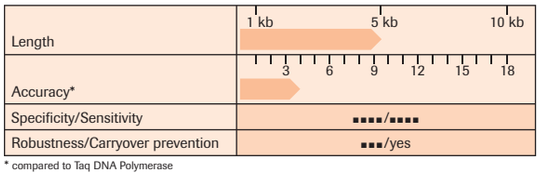


Figure 9: Varying amounts of human genomic DNA were used for the amplification of a 4.8-kb fragment from the tPA gene.
Result: The FastStart High Fidelity PCR System showed superior sensitivity and specificity compared to enzymes from two other suppliers.

Product	Cat. No.	Pack Size
FastStart High Fidelity PCR System ▼	03 553 426 001	125 units
	03 553 400 001	2 x 250 units
	03 553 361 001	10 x 250 units
PCR Optimization Kit *	11 636 138 001	1 kit

For all dNTPacks, which provide the single reagent and PCR-Grade nucleotides refer to page 29.



* compared to Taq DNA Polymerase

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Difficult Template PCR

Conquer GC-rich templates

GC-RICH PCR System

Choose the GC-RICH PCR System, a blend of a proofreading polymerase and Taq DNA Polymerase to power through templates that are difficult or impossible to amplify with other polymerases, other blends of polymerases or additives. The enhanced processivity of the blend and the unique GC-RICH Resolution Solution are combined to deliver superior performance.

- **Amplify fragments up to 5 kb.**
  The reagent enables the amplification of a variety of DNA and cDNA fragments.
- **Work with difficult DNA templates.**
  The optimally designed system can amplify difficult templates, including GC-rich targets, repetitive sequences, as well as uniform amplification of a mixture of nucleic acids with varying GC content.
- **Benefit from PCR-Grade Water.**
  The GC-RICH PCR System conveniently provides a GC-RICH Resolution Solution and buffers, with and without Mg, Mg-solution, and PCR-Grade Water.

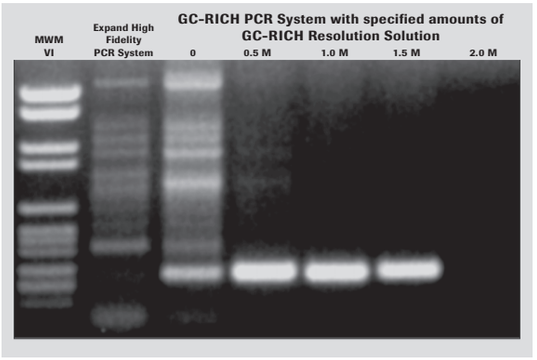

Figure 10: Successfully amplify GC-rich templates with the GC-RICH PCR System.
Amplification of a 264 bp template (74% GC content) within the human ApoE gene using the GC-RICH PCR System or the Expand High Fidelity PCR System.
Result: The GC-RICH PCR System amplifies the GC-rich fragment with high specificity and yield using GC-RICH Resolution Solution.

Product	Cat. No.	Pack Size
GC-RICH PCR System ▼	12 140 306 001	100 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.

Length	1 kb – 5 kb – 10 kb
Accuracy*	3 – 6 – 9 – 12 – 15 – 18
Specificity/Sensitivity	▪▪▪/▪▪▪
Robustness/Carryover prevention	▪▪▪▪/no

* compared to Taq DNA Polymerase

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High Fidelity PCR

Proofreading for every high-fidelity application

Pwo SuperYield DNA Polymerase

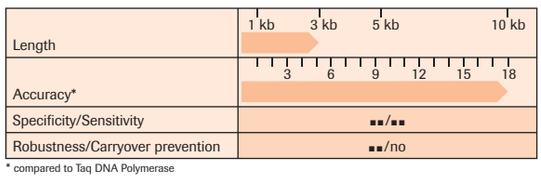
Utilize Pwo SuperYield DNA Polymerase to yield considerably high amounts of PCR product with constant high fidelity. This product delivers superior results due to the recombinant enzyme Pwo DNA Polymerase and a newly optimized buffer system. It amplifies fragments up to 3 kb – from easy templates even longer amplicons are possible.

- **Insist on excellent fidelity.**
  This enzyme yields 18-fold higher fidelity compared to Taq DNA Polymerase.
- **Get highest yields.**
  The robust buffer system facilitates outstanding yields without optimization.
- **Achieve peak performance – even with difficult templates.**
  Pwo SuperYield DNA Polymerase comes with the GC-RICH Solution for the amplification of difficult targets like GC-rich DNA.

Figure 11: Amplification of a 1.7 kb fragment of the tPA exon from human genomic DNA.
Result: Pwo SuperYield DNA Polymerase showed highest yield and consistency compared to enzymes from two other suppliers.

Product	Cat. No.	Pack Size
Pwo SuperYield DNA Polymerase ▼,◆	04 340 868 001	100 units
	04 340 850 001	2 x 250 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.

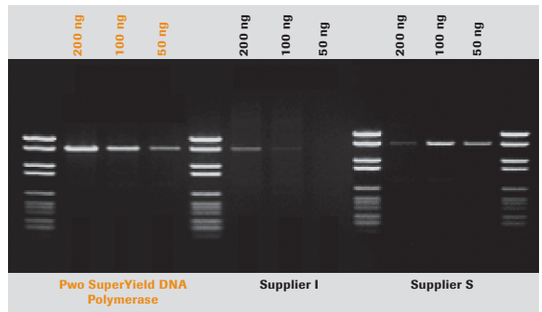


* compared to Taq DNA Polymerase

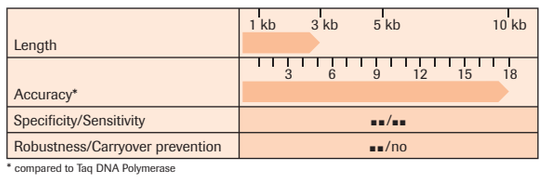
Pwo Master

Just add template, primers and PCR-Grade Water to Pwo Master and get results with less effort and minimal risk of contamination. Pwo Master is a ready-to-use double-concentrated master mix containing polymerase, buffer and nucleotides.

- **Maximize convenient handling.**
  Ready-to-use Pwo Master reduces handling steps to a minimum.
- **Increase fidelity.**
  The mix shows an approximate 18 times higher fidelity compared to Taq DNA Polymerase.
- **Reduce setup time.**
  Pwo Master can be stored at +2 to +8°C – ready for immediate use – allowing you to start the PCR immediately without time-consuming thawing of reagents.



Product	Cat. No.	Pack Size
Pwo Master ▼,◆	03 789 403 001	2.5 ml (10 x 250 µl)



* compared to Taq DNA Polymerase

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High Fidelity PCR

Combine high yields, accuracy, and the prevention of carryover contamination

Expand High Fidelity^{PLUS} PCR System

Use this enzyme blend to amplify fragments up to 5 kb from all types of DNA with outstanding yield and fidelity. In addition, this enzyme blend also incorporates dUTP, and, in combination with Uracil-DNA Glycosylase, can be used to safeguard PCR reactions from cross contamination. Expand High Fidelity^{PLUS} PCR System is a next-generation blend consisting of Taq DNA Polymerase, and a novel proofreading protein isolated and characterized by Roche Applied Science.

- **Get six-fold higher fidelity and higher yields.** The enzyme blend shows a significantly higher fidelity compared to Taq DNA Polymerase and is the ideal tool for high-fidelity applications (*e.g.*, cloning).
- **Safeguard PCR from cross contamination.** The Expand High Fidelity^{PLUS} PCR System is the product of choice if a larger number of targets need to be amplified at the same time.
- **Efficiently label DNA.** The product can be used for the labeling of DNA fragments with radioactive or non-radioactive modified nucleotides.

Carryover contamination, in which the product of a previous PCR erroneously serves as a template in a subsequent reaction, is a problem in every laboratory. See page 33 of this brochure for more details.

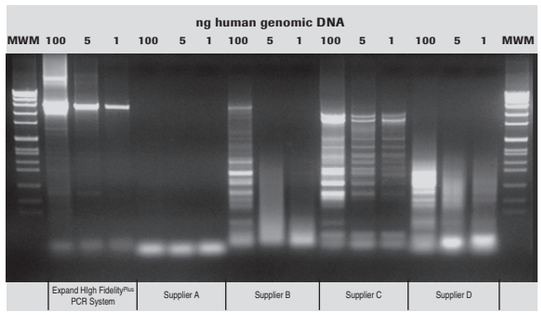

Figure 12: Comparison of Expand High Fidelity^{PLUS} PCR System with four commercially available polymerase mixes. Various amounts (in ng) of human genomic DNA were used to amplify a 4.8-kb fragment from the tissue plasminogen activator (tPA) gene, in accordance with each manufacturer's recommended conditions.
Supplier A: Mixture of Taq DNA polymerase (deleted at N-terminus), a proofreading polymerase, and a hot start antibody.
Supplier B and D: Mixture of Taq DNA polymerase and a proofreading polymerase.
Supplier C: Mixture of Taq DNA polymerase, a proofreading polymerase, and an enhancing factor.
Result: Expand High Fidelity^{PLUS} PCR System produced the best specificity, sensitivity, and yield, even from as little as 1 ng human genomic DNA.

Product	Cat. No.	Pack Size
Expand High Fidelity^{PLUS} PCR System ▼	03 300 242 001	125 units
	03 300 226 001	2 x 250 units
	03 300 234 001	10 x 250 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.

Length	1 kb — 5 kb — 10 kb
Accuracy*	3 6 9 12 15 18
Specificity/Sensitivity	▪▪▪/▪▪▪
Robustness/Carryover prevention	▪▪▪/yes

* compared to Taq DNA Polymerase

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High Fidelity PCR

Maximize convenience for longer fragments

High Fidelity PCR Master

Use the convenient High Fidelity PCR Master: a double-concentrated, ready-to-use mix, which combines Expand High Fidelity PCR System with PCR-Grade dNTPs, $MgCl_2$, and an optimized reaction buffer. The High Fidelity PCR Master delivers twice the yield and three times better fidelity than Taq DNA Polymerase alone.

- **Amplify longer fragments.**
  The High Fidelity PCR Master allows the amplification of a variety of DNA and cDNA fragments up to 5 kb.
- **Increase fidelity.**
  The mix shows an approximate three times higher fidelity compared to Taq DNA Polymerase.
- **Reduce setup time.**
  High Fidelity PCR Master can be stored at +2 to +8°C – ready for immediate use – without time-consuming thawing of reagents.
- **Benefit from PCR-Grade Water.**
  Besides the double-concentrated master mix, the High Fidelity PCR Master provides PCR-Grade Water.
- **Profit from minimal contamination risk.**
  Only two pipetting steps are required – addition of primers and template.

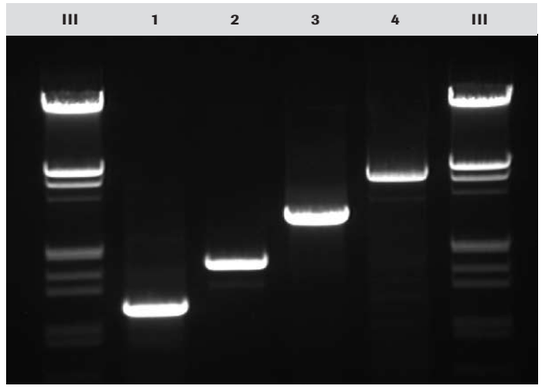
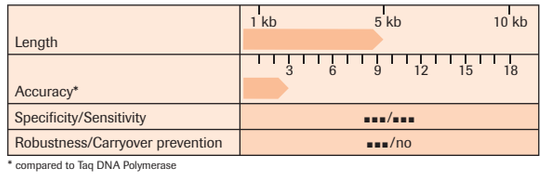


Figure 13: Performance of the High Fidelity PCR Master. Four different templates were amplified from human genomic DNA with the High Fidelity PCR Master according to instructions given in the package inserts.

MWM III: Molecular Weight Marker III
Lane 1: 1.1 kb fragment of the collagen gene
Lane 2: 1.7 kb fragment of the t-PA gene
Lane 3: 2.9 kb fragment of the p53 gene
Lane 4: 4.8 kb fragment of the t-PA gene

Result: The High Fidelity PCR Master can amplify different targets without individual modification of reaction conditions or reagents. The results show fragments ranging from 1.1 kb up to 4.8 kb can be obtained with high yield and specificity.

Product	Cat. No.	Pack Size
High Fidelity PCR Master ▼	12 140 314 001	5 ml (10 x 0.5 ml)



* compared to Taq DNA Polymerase

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Long Template PCR

Amplify longer fragments

Expand Long Template PCR System

Choose Expand Long Template PCR System for long and accurate PCR. This optimized enzyme blend and three-buffer set generates PCR products from 5 to 20 kb in length.

- **Amplify longer templates than ever before.** This optimized enzyme blend and specially developed three-buffer set generate PCR products from 5 to 20 kb in length from complex genomic DNA.
- **Achieve higher yields and fidelity.** The Expand Long Template PCR System delivers three times the fidelity and much higher yields compared to Taq DNA Polymerase.
- **Improve PCR efficiency.** More full-length product is obtained when characterizing human gene loci, fingerprinting DNA, or isolating entire genes from cDNA or entire viral genomes.

Product	Cat. No.	Pack Size
Expand Long Template PCR System ▼	11 681 834 001	150 units
	11 681 842 001	2 x 360 units
	11 759 060 001	10 x 360 units

Length	5 kb – 20 kb – 25 kb
Accuracy*	3 6 9 12 15 18
Specificity/Sensitivity	▪▪▪/▪▪
Robustness/Carryover prevention	▪▪▪/no

* compared to Taq DNA Polymerase

A crucial component of obtaining long PCR products is the quality (length and purity) of the initial template. Care should be taken when handling and preparing the genomic DNA template. The High Pure Template Preparation Kit is recommended for this application (see page 8).

Expand 20 kb^{PLUS} PCR System

Select Expand 20 kb^{PLUS} PCR System when amplifying fragments even longer than 20 kb. Leave behind the product-length limits of standard PCR in advanced applications (*e.g.*, inter-Alu PCR and RT-PCR). The Expand 20 kb^{PLUS} PCR System is a powerful enzyme mix containing Taq DNA Polymerase, and a thermostable polymerase with proofreading activity.

- **Go to the limit.** Expand 20 kb^{PLUS} PCR System has specifically optimized buffer and enzyme-blend mixture to amplify extra-long pieces of DNA – over 20 kb.
- **Easily monitor amplification.** For control reactions, the Expand 20 kb^{PLUS} PCR System provides human genomic DNA and human control β-globin primers which allow the amplification of a 23 kb fragment.
- **Test your template quality.** The reagents of the control reaction can also be used to test the quality of human template DNA and/or the respective primer pairs.

Product	Cat. No.	Pack Size
Expand 20 kb^{PLUS} PCR System ▼	11 811 002 001	200 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.

Length	15 kb – 20 kb – 35 kb
Accuracy*	3 6 9 12 15 18
Specificity/Sensitivity	▪▪/▪▪
Robustness/Carryover prevention	▪▪▪/no

* compared to Taq DNA Polymerase

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Basic PCR

GMP-Grade – benefit from the high-quality standards

Taq DNA Polymerase, GMP Grade

Achieve consistent results with our validated Taq DNA Polymerase, GMP Grade. Taq DNA Polymerase, GMP Grade belongs to the family of high-performance amplification enzymes supplied by Roche Applied Science and is manufactured using validated production, quality control, and filling procedures.

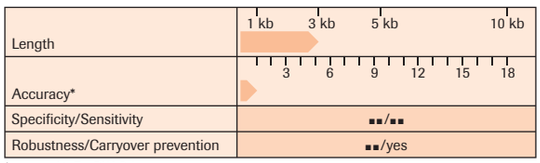
- **Minimize risk of interference.**
  Due to stringent quality control, product segregation, and GMP manufacturing, a contamination threat is minimized.
- **Enjoy outstanding lot-to-lot consistency.**
  All manufacturing processes are validated, clearly defined, systematically reviewed, and shown to be capable of consistently manufacturing products of the required quality, and complying with their specifications.
- **Insist on sophisticated manufacturing.**
  The production areas are access controlled and class 100 000 with reference to the non-viable particle counts (< 0.5 µm) and less than 200 cfu/m³. The filling of bulk solutions takes place in a laminar flow box.
- **Utilize most stringent quality tests.**
  Quality control includes Bioburden (≤50 cfu/ml) and performance-tests on human genomic and plasmid DNA using the LightCycler® Instrument.
- **Try convenient packaging options.**
  To meet your research needs, Taq DNA Polymerase, GMP Grade comes with 10x concentrated PCR buffer including $MgCl_2$ in 5 ml vials.

In view of the current and future importance of "good manufacturing practice" (GMP), our GMP-Grade Taq DNA Polymerase meets the high quality and documentation requirements of research and development in the pharmaceutical and biotechnology industries, while providing convenient packaging options.

Benefit from the high-quality standards provided by GMP

The general rules for "good manufacturing practice" (GMP) were established for regulated products by FDA. Manufacturers of raw materials are also encouraged, although not required to follow these guidelines. In an attempt to ease the transitions between research, development, scale-up, and production, Roche Applied Science began manufacturing and bottling GMP-grade products according to these guidelines.

Product	Cat. No.	Pack Size
Taq DNA Polymerase, GMP Grade ▼	03 734 927 001	1,000 units
	03 734 935 001	5,000 units



* compared to Taq DNA Polymerase

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Basic PCR

Consistent results lot-to-lot

Taq DNA Polymerase

Achieve consistent results in simple, routine polymerase chain reactions by using Roche Applied Science's Taq DNA Polymerase, which is held to our rigorous purity and quality standards. Before it arrives in your laboratory, every lot of Taq DNA Polymerase passes several stringent tests for functionality, and purity ensuring reliable, consistent results with every lot.

- **Obtain reliable, reproducible results.**
  Roche Applied Science's Taq DNA Polymerase exemplifies lot-to-lot consistency (Figure 14) – tube-to-tube, experiment-to-experiment.
- **Eliminate testing of each new lot.**
  Taq DNA Polymerase is rigorously tested resulting in highest lot-to-lot consistency.

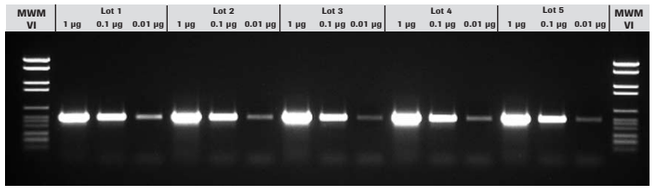

Figure 14: Lot-to-lot consistency ensures reproducible results. Five different lots of Taq DNA Polymerase were tested for the ability to amplify a 0.5-kb fragment of lambda DNA. **Result:** Reliable, consistent results were obtained with every lot of Roche Applied Science's Taq DNA Polymerase that was tested.

Product	Cat. No.	Pack Size
Taq DNA Polymerase, 1 unit/µl ▼	11 647 679 001	250 units
	11 647 687 001	4 x 250 units
Taq DNA Polymerase, 5 units/µl ▼	11 146 165 001	100 units
	11 146 173 001	2 x 250 units
	11 418 432 001	4 x 250 units
	11 596 594 001	10 x 250 units
	11 435 094 001	20 x 250 units

For all dNTPacks which provide the single reagent and PCR-Grade nucleotides, refer to page 29.

Length	1 kb 3 kb 5 kb 10 kb
Accuracy*	3 6 9 12 15 18
Specificity/Sensitivity	▪▪/▪▪
Robustness/Carryover prevention	▪▪/yes

* compared to Taq DNA Polymerase

PCR Master

Choose the PCR Master which comprises all the reagents needed to perform standard PCR (except template and primer) in a double-concentrated, ready-to-use mix.

- **Maximize convenience and experimental consistency.**
  This ready-to-use master mix only requires the addition of primers and template.
- **Eliminate thawing steps.**
  The master can be stored at 4°C and can be used immediately.
- **Reduce contamination risk.**
  Adding template and primer to the master necessitates only two pipetting steps.

Product	Cat. No.	Pack Size
PCR Master ▼,●	11 636 103 001	10 x 0.5 ml

Length	1 kb 2 kb 5 kb 10 kb
Accuracy*	3 6 9 12 15 18
Specificity/Sensitivity	▪▪/▪▪
Robustness/Carryover prevention	▪▪/no

* compared to Taq DNA Polymerase

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PCR Enzyme dNTPacks

Ensure optimal performance by using reagents combined with PCR-Grade Nucleotides

Choose Roche Applied Science's dNTPacks, convenient products that combine PCR-Grade Nucleotides, thermostable enzymes and enzyme blends, and all associated components such as buffers and PCR-enhancing additives. Our PCR-Grade Nucleotides are assayed for function in RT-PCR, ensuring optimal performance of all components. Each dNTPack contains the additive-free sodium salt nucleotides as a ready-to-use mix (10 mM of each dNTP).

- **Profit from best performance.**
  Superior enzymes, combined with a mix of ultrapure PCR-Grade Nucleotides, ensure highest sensitivity and performance of amplification reactions.
- **Safeguard your precious reaction components.**
  The extensive investment in generating template material should not be risked by using nucleotides from another supplier.
- **Simplify ordering.**
  dNTPacks provide everything you need for PCR in one convenient package.
- **Benefit from an attractive price.**
  Thermostable DNA polymerases and premixed solutions of PCR-Grade Nucleotides are provided in one economical package.

Rely on dNTPs that are free of PCR inhibitors.

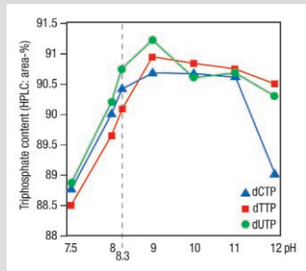
The enzymatic synthesis process avoids the generation of contaminants like tetraphosphate, which can disintegrate and form pyrophosphate – a known inhibitor of amplification reactions.

Insist on patented technology.

PCR-Grade Nucleotides are manufactured by an enzymatic synthesis process that does not generate nucleotides with modified bases, tetraphosphates, or pyrophosphate contaminants common in chemically synthesized nucleotide preparations. The patented buffer conditions of Roche's PCR-Grade Nucleotides result in nucleotide solutions with unmatched stability values and long shelf life (Figure 15).

Optimum pH for dNTPs 100 mmol/l Na^+ solutions were stressed. Purity was checked using HPLC.

dCTP, dTTP, and dUTP: 28 days at 35°C



dATP and dGTP: 28 days at 35°C

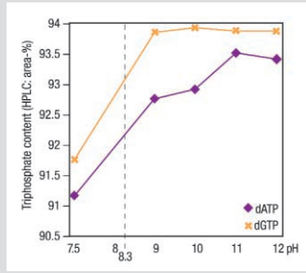


Figure 15: PCR-Grade Nucleotide stability experiment. Nucleotides were stored at 35°C for indicated time periods at indicated pH values. Stability of purines and pyrimidines is improved when nucleotides are stored at pH 8.3 instead of lower pH storage conditions. Stability tests also indicate enhanced tolerance of increasing pH through normal experimental pH values.

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PCR Enzyme dNTPacks

Ensure optimal performance by using reagents combined with PCR-Grade Nucleotides

Insist on nucleotides that are extensively function-tested.

Rest assured that our PCR-Grade Nucleotides will work well in your lab, because they are function-tested in RT-PCR to confirm suitability for PCR and reverse transcription at the same time. Each lot is also tested for the absence of RNases, DNases, and nicking activity to protect your precious template.

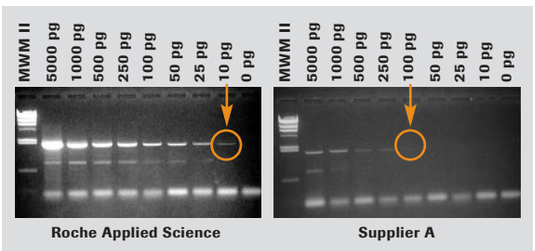

Figure 16: RT-PCR of a 1849-bp fragment of the human dystrophin gene.
Varying amounts of human skeletal muscle total RNA were used in the RT-PCR.
Result: Roche's dNTPs produced the RT-PCR product from only 10 picograms of RNA, compared with 100 picograms when using Supplier A's dNTPs (10-fold higher sensitivity with Roche's dNTPs).

RT-PCR testing covers interferences caused by RNAses, DNAses, tetra- and pyrophosphates, nucleobase modifications, nucleases, proteases, PCR-interfering cations and other possible impurities. RT-PCR functional test confirms suitability for PCR and reverse transcription at the same time.

Improve the performance and consistency of every PCR.

Improve experimental consistency by choosing nucleotides that always exhibit a consistent purity of >99% dNTP and <0.9% dNDP as determined by HPLC (Figure 17).

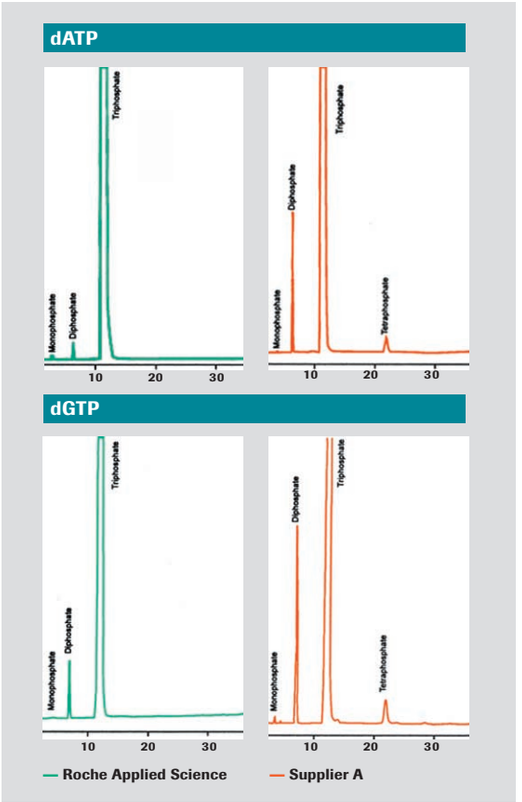

Figure 17: HPLC (RP 18) purity analysis of Roche's PCR-Grade Nucleotides in comparison with Supplier A's nucleotides.
Result: Roche's PCR-Grade Nucleotides are free of tetraphosphates and show much higher purity overall in comparison to Supplier A's nucleotides.

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PCR Enzyme dNTPacks

Ensure the highest purity.

PCR-Grade Nucleotides are stable for at least 30 months after the production date when stored at –20°C.

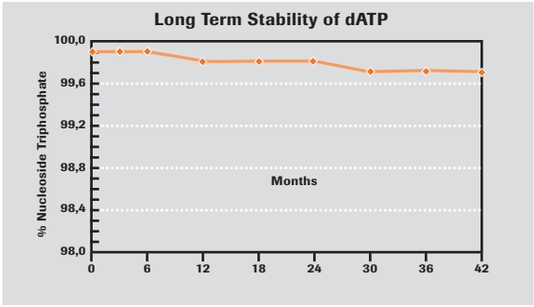

Figure 18: Long term stability of dATP at -20°C.
Result: Even after storage for 42 months at -20°C, PCR-Grade dATP contains 99.7% of the nucleoside triphosphate.

Product	Cat. No.	Pack Size
FastStart Taq DNA Polymerase dNTP Pack ▼	04 738 314 001	100 units
	04 738 357 001	500 units (2 x 250 units)
	04 738 381 001	1,000 units (4 x 250 units)
	04 738 403 001	2,500 units (10 x 250 units)
	04 738 420 001	5,000 units (20 x 250 units)
FastStart High Fidelity PCR System dNTPack ▼	04 738 284 001	125 units
	04 738 292 001	500 units (2 x 250 units)
	04 738 306 001	2,500 units (10 x 250 units)
Expand 20 kb^{PLUS} PCR System, dNTPack ▼	04 743 814 001	200 units
Expand High Fidelity PCR System, dNTPack ▼	04 738 250 001	100 units
	04 738 268 001	500 units (2 x 250 units)
	04 738 276 001	2,500 units (10 x 250 units)
Expand High Fidelity^{PLUS} PCR System, dNTPack ▼	04 743 725 001	125 units
	04 743 733 001	500 units (2 x 250 units)
	04 743 374 001	2,500 units (10 x 250 units)
GC-RICH PCR System, dNTPack ▼	04 743 784 001	100 units
Pwo SuperYield DNA Polymerase, dNTPack ▼,◆	04 743 750 001	100 units
	04 743 776 001	500 units (2 x 250 units)
Taq DNA Polymerase, dNTPack ▼	04 728 866 001	100 units
	04 728 874 001	500 units
	04 728 882 001	(2 x 250 units)
	04 728 904 001	1,000 units
	04 728 858 001	(4 x 250 units)
		2,500 units (10 x 250 units)
		5,000 units (20 x 250 units)
Taq DNA Polymerase (1 U/µl), dNTPack ▼	04 738 225 001	250 units
	04 738 241 001	1,000 units (4 x 250 units)

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Nucleotide Mixes, Sets and Single Nucleotides

Ensure optimal performance

PCR Nucleotide Mix

Add the PCR Nucleotide Mix directly to amplification reactions. This ready-to-use nucleotide mix is a premixed solution of multiple PCR-Grade nucleotide sodium salts in water: dATP, dGTP, dCTP, and dTTP (each at a concentration of 10 mM). This mix is optimized for use in all types of amplification reactions and primer extension reactions.

Product	Cat. No.	Pack Size
PCR Nucleotide Mix *	11 581 295 001	200 µl (reaction volume)
	11 814 362 001	10 x 200 µl

Deoxynucleoside Triphosphate Set, PCR-Grade

Use the PCR-Grade Deoxynucleoside Triphosphate Set in all types of PCR amplification reactions and primer-extension reactions, and enjoy the flexibility of single reagents. It contains 4 individual vials of dATP, dCTP, dGTP, and dTTP, each as a 100 mM solution of the Na-salt.

Product	Cat. No.	Pack Size
Deoxynucleoside Triphosphate Set, PCR- Grade *	11 969 064 001	4 x 25 µmol
	03 622 614 001	4 x 125 µmol

PCR Nucleotide Mix^{PLUS}

Prevent carryover contamination from previous amplifications. The incorporation of dUTP in place of dTTP allows the degradation of contaminating PCR products from previous reactions, using Uracil-DNA Glycosylase (UNG). This ready-to-use nucleotide mix is a premixed solution of the sodium salts of dATP, dGTP, and dCTP, each at a concentration of 10 mM, and dUTP at a concentration of 30 mM in water.

Product	Cat. No.	Pack Size
PCR Nucleotide Mix^{PLUS} *	11 888 412 001	2 x 100 µl

Single Nucleotides

Nucleotides		
Product	**Cat. No.**	**Pack Size**
dATP, PCR-Grade *	11 934 511 001	25 µmol
	11 969 013 001	125 µmol
	03 732 681 001	4 x 125 µmol
dCTP, PCR-Grade *	11 934 520 001	25 µmol
	11 969 021 001	125 µmol
	03 732 690 001	4 x 125 µmol
dGTP, PCR-Grade *	11 934 538 001	25 µmol
	11 969 030 001	125 µmol
	03 732 703 001	4 x 125 µmol
dTTP, PCR-Grade *	11 934 546 001	25 µmol
	11 969 048 001	125 µmol
	03 732 711 001	4 x 125 µmol
dUTP, PCR-Grade *	11 934 554 001	25 µmol
	11 969 056 001	125 µmol
	03 732 720 001	4 x 125 µmol

For detailed information about our single nucleotides, refer to our PCR Special Interest Site at **www.roche-applied-science.com/pcr**

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Ribonucleotides, Dideoxynucleotides and Modified Nucleotides

Ensure optimal performance

Insist on the highest quality nucleotides for all your daily applications. Roche Applied Science offers you optimized products for *in-vitro* transcription, *in-vitro* synthesis of DNA and RNA, sequencing, synthesis of hemi-methylated DNA and DNA labeling.

Ribonucleoside Triphosphate Set

Use the Ribonucleoside Triphosphate Set for *in vitro* transcription reactions and RNA polymerase-directed DNA sequencing. It contains 4 individual vials of ATP, CTP, GTP, and UTP – each as a 100 mM solution of the Li-salt. Single ribonucleotides are also available.

Product	Cat. No.	Pack Size
Ribonucleoside Triphosphate Set	11 277 057 001	4 x 20 µmol (4 x 200 µl)
ATP, special quality for molecular biology	11 140 965 001	40 µmol (400 µl)
CTP, special quality for molecular biology	11 140 922 001	40 µmol (400 µl)
GTP, special quality for molecular biology	11 140 957 001	40 µmol (400 µl)
UTP, special quality for molecular biology	11 140 949 001	40 µmol (400 µl)

7-Deaza-dGTP

Use 7-Deaza-dGTP instead of dGTP in primer extension reactions (*e.g.*, sequencing according to Sanger) and receive a better resolution of GC-rich regions. 7-Deaza-dGTP contains 7-deaza-2'-deoxy-guanosine-5'-triphosphate as a 10 mM solution of the Li-salt.

Product	Cat. No.	Pack Size
7-Deaza-dGTP ▲	10 988 537 001	2 µmol (200 µl)

Dideoxynucleoside Triphosphate Set, Sequencing-Grade

Use Dideoxynucleoside Triphosphate Set in sequencing and cycle-sequencing reactions. The Dideoxynucleoside Triphosphate Set contains 4 individual vials of ddATP, ddCTP, ddGTP, and ddTTP, each as a 10 mM solution of the Na-salt in water. Sequencing-Grade Dideoxynucleotides are optimized for use in sequencing and cycle-sequencing reactions.

Product	Cat. No.	Pack Size
Dideoxynucleoside Triphosphate Set, Sequencing-Grade	03 732 738 001	4 x 1 µmol (4 x 100 µl)

5'-Methyl-2'-deoxycytidine-5'-triphosphate

Use 5'-methyl-2'-deoxy-cytidine-5'-triphosphate for the *in vitro* synthesis of hemi-methylated DNA.

Product	Cat. No.	Pack Size
5'-Methyl-2'-deoxy-cytidine-5'-triphosphate	10 757 047 001	10 µmol

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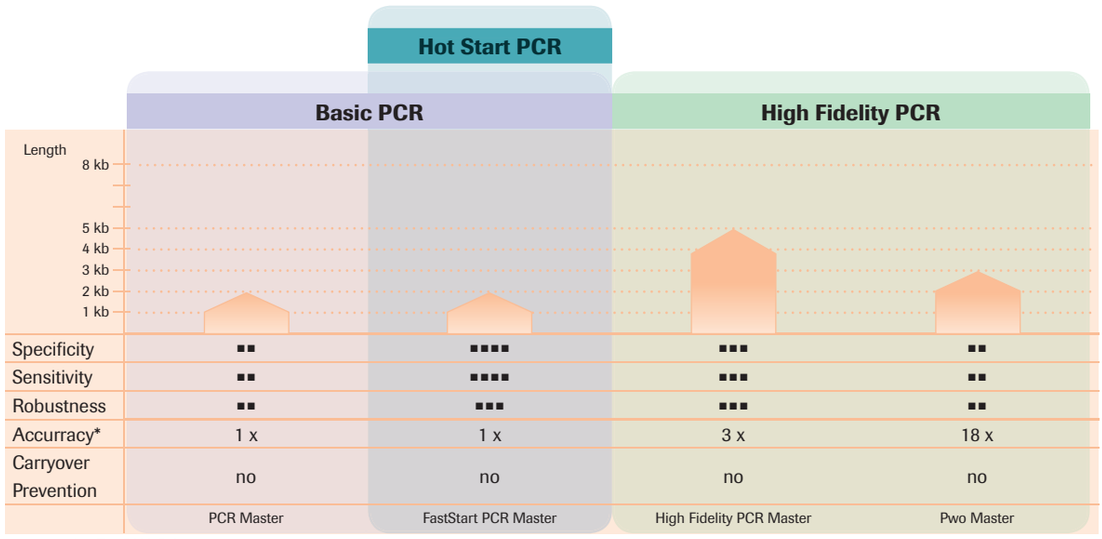
Master Mixes

Maximize convenience

Combine all the benefits of our amplification products with all the benefits of our PCR-Grade Nucleotides in an ultimate convenient format. Roche Applied Science's Master mixes are ready-to-use, double-concentrated solutions that contain all the reagents (except PCR primers and template) needed for running PCR: Polymerase or polymerase blend, magnesium chloride, double-concentrated reaction buffer, and nucleotides (dATP, dCTP, dGTP, dTTP, 0.4 mM each).

- **Simplify PCR setup.**
  Only two pipetting steps are needed before starting PCR.
- **Minimize the risk of contamination.**
  The ready-to-use reaction mix reduces potential contacts to contamination sources.
- **Enjoy convenient packaging options.**
  Master mixes can be obtained in 50 ml quantities designed for high throughput applications, or in multiple vials containing smaller volumes.
- **Save valuable time.**
  No thawing required because of 4°C stability.
- **Insist on room temperature stability.**
  Roche Applied Science's master mixes are compatible with robotics pipetting stations because of tested stability at room temperature.



* compared to Taq DNA Polymerase

Product	see page
FastStart PCR Master	18
High Fidelity PCR Master	23

Product	see page
PCR Master	26
Pwo Master	31

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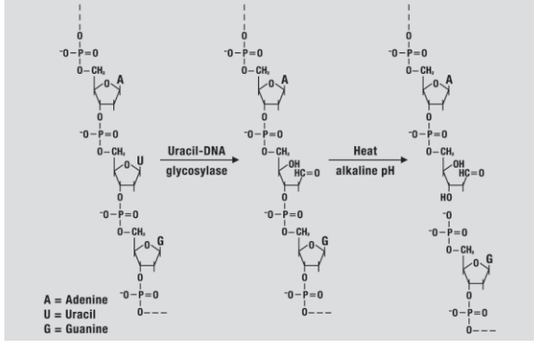
Products for the Prevention of Carryover Contamination

Safeguard your PCR

Avoid carryover contamination between PCRs to eliminate a source of false positives. Carryover contamination, in which the product of a previous PCR erroneously serves as a template in a subsequent reaction, is a problem in every laboratory. Effective carryover prevention does not have to be difficult. Roche Applied Science offers a variety of easy-to-apply products that enzymatically and chemically degrade specific PCR products from previous amplifications, but do not degrade native DNA templates or primers. Procedures to prevent carryover contamination involve:

1. **Substituting dUTP for dTTP during PCR amplification**
   Uracil-containing DNA (U-DNA) can be produced with one of the enzymes listed in the table below.
2. **Treating PCR mixtures with Uracil-DNA Glycosylase (UNG)**
   Prior to amplification the reaction mix is incubated with UNG and apyrinic sites are created.
3. **Cleaving at an elevated temperature (95°C)**
   Under alkaline conditions (during the initial denaturation step) apyriminic polynucleotides are cleaved leading to removal of contaminating template DNA (Figure 19).
4. **Inactivating UNG**
   UNG acitivity is removed in as little as 2 minutes by heating at 95°C (for the heat-labile version).



Choose from the following high-quality amplification enzymes to introduce dUTP during amplification. Used in combination with the associated products, they will prevent false-positive results caused by carryover contamination.

Product	Cat. No.	Pack Size
Expand High Fidelity^{PLUS} PCR System	see page 22	
FastStart Taq DNA Polymerase	see page 17	
FastStart High Fidelity PCR System	see page 19	
Taq DNA Polymerase	see page 26	
PCR Core Kit^{PLUS} ▼, §	11 585 541 001	1 kit (50 reactions)
Tth DNA Polymerase +	11 480 014 001 11 480 022 001	100 units 2 x 250 units
C. therm. Polymerase One-Step RT-PCR System +, ‡	12 016 338 001 12 016 346 001	50 reactions 250 reactions

Associated Product	Cat. No.	Pack Size
Uracil-DNA Glycosylase (UNG), heat-labile *, §	11 775 367 001 11 775 375 001	100 units 500 units
Uracil-DNA Glycosylase (UNG) *, §	11 444 646 001	100 units
PCR Nucleotide Mix^{PLUS} *	11 888 412 001	2 x 100 µl
dUTP, PCR-Grade *	11 934 554 001 11 969 056 001 03 732 720 001	25 µmol (250 µl) 125 µmol (1,250 µl) 4 x 125 µmol (4 x 1,250 µl)

Figure 19: Hydrolysis of uracil-glycosidic bonds at U-DNA sites and cleavage with alkaline pH and heat.

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Labeling of Nucleic Acids

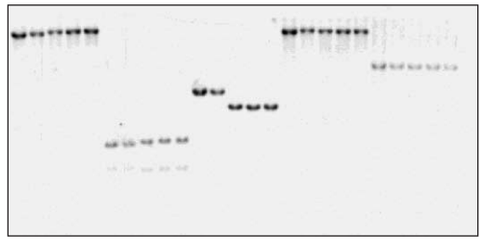
Experience safe nonradioactive labeling

Choose from a wide variety of products for nonradioactive labeling of nucleic acids by PCR, RT-PCR or primer extension available from Roche Applied Science. The DIG labeling method with digoxigenin is based on a steroid isolated from digitalis plants. The DIG-labeled nucleotides may be incorporated enzymatically into nucleic acids by DNA polymerases as well as RNA polymerases. It allows the safe and efficient labeling of DNA and RNA, or oligonucleotide probes.

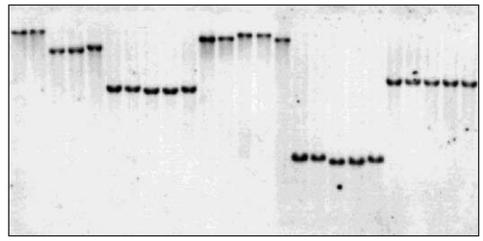
- **Avoid handling of radioactive compounds.** The DIG system employs nonradioactive steroid hapten digoxigenin.
- **Insist on high sensitivity.** It is more sensitive than radioactive methods.
- **Save time.** Unlike other methods, this system requires short exposure times, in the range of minutes rather than hours or days.
- **Put safety first.** The system omits any contact with hazardous materials, and avoids environmental contamination.
- **Achieve high reproducibility.** Probes are stable for a minimum of one year and thus are reusable repeatedly.
- **Rely on well-established protocols.** For nonradioactive detection of nucleic acids, Roche Applied Science provides a plethora of

The DIG System is the nonradioactive technology of choice to label and detect nucleic acids for multiple high sensitive applications such as *in situ* hybridization, or filter hybridization. Roche Applied Science was one of the first companies to offer a nonradioactive technology allowing customers to move away from the use of hazardous radioactive isotopes.

1. Hybridization Probe: A



2. Hybridization Probe: B



3. Hybridization Probe: C

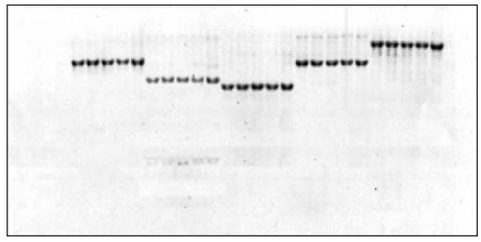


Figure 20: Stripping and reprobing of Southern blots. DNA (10 µg) of 5 different barley cultivars was digested with *Eco*R I, *Hin*d III, *Ssp* I, *Xba* I, separated by gel electrophoresis and blotted on membrane. The probe was labeled by PCR and refers to a single-copy DNA sequence of barley. For the detection of the corresponding restriction fragments, 2 µl PCR product/ml and DIG EASY HYB were used with an incubation temperature of 38°C o.n. Films were exposed for 45 – 60 minutes using CSPD as substrate. Stringent washes allowed the reprobing of the same membrane.
Result: PCR-labeled probes show highest specificity and sensitivity. The same membrane was stripped and reprobed several times.

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The DIG System uses an adapted immunochemical technique. The preferred detection method for DIG-labeled probes is incubation with a chemiluminescent substrate like CDP *Star* or CSPD, and subsequent exposure to X-ray film. PCR is also an excellent tool for labeling DNA that can be used in a variety of applications.
Biotin can be incorporated and used in the same way as digoxigenin. It can be detected by anti-biotin antibodies. However, streptavidin or avidin is more frequently used because these molecules have a high binding capacity for biotin.
Fluorescein-dUTP/UTP/ddUTP can be incorporated enzymatically into nucleic acids. Since fluorescein is a direct label, no immunocytochemical visualization procedure is necessary, and exhibits low background. Other fluorochrome-labeled nucleotides, such as Tetramethylrhodamine-dUTP are also available from Roche Applied Science.

Amplification products for Labeling

Product	see page
Taq DNA Polymerase	26
FastStart Taq DNA Polymerase	17
FastStart High Fidelity PCR System	19
Expand High Fidelity^{PLUS} PCR System	22
Pwo SuperYield DNA Polymerase	21
Transcriptor Reverse Transcriptase	12

Single Nucleotides for Labeling of RNA

Product	Cat. No.	Pack Size
Digoxigenin-11-UTP	11 209 256 910	250 nmol (25 µl) 10 mM
	03 359 247 910	200 nmol (57 µl) 3.5 mM
Biotin-16-UTP	11 388 908 910	250 nmol (25 µl)
Fluorescein-12-UTP	11 427 857 910	250 nmol (25 µl)

For DNA Molecular Weight Marker, DIG-labeled see page 38.

Mixes for Labeling of DNA

Product	Cat. No.	Pack Size
DIG DNA Labeling Mix ◇	11 277 065 910	50 µl
PCR DIG Labeling Mix *, ◇	11 585 550 910	2 x 250 µl
PCR DIG Labeling Mix^{PLUS} *, ◇	11 835 289 910	2 x 250 µl
PCR Fluorescein Labeling Mix *, ◇	11 636 154 910	100 µl (10 PCRs)

Mixes for Labeling of RNA

Product	Cat. No.	Pack Size
DIG RNA Labeling Mix	11 277 073 910	40 µl
Biotin RNA Labeling Mix	11 685 597 910	40 µl
Fluorescein RNA Labeling Mix	11 685 619 910	40 µl (20 reactions)

Single Nucleotides for Labeling of DNA

Product	Cat. No.	Pack Size
Digoxigenin-11-dUTP, alkali-stable ×	11 093 088 910	25 nmol (25 µl)
	11 558 706 910	125 nmol (125 µl)
	11 570 013 910	5 x 125 nmol (5 x 125 µl)
Digoxigenin-11-dUTP, alkali-labile ◇	11 573 152 910	25 nmol (25 µl)
	11 573 179 910	125 nmol (125 µl)
Biotin-16-dUTP	11 093 070 910	50 nmol (50 µl)
Fluorescein-12-dUTP	11 373 242 910	25 nmol (25 µl)
Tetramethyl-rhodamine-5-dUTP	11 534 378 910	50 nmol (50 µl)

For a detailed overview of the wide variety of kits and individual reagents for the labeling and detection of nucleic acids by different methods, visit our Special Interest Site:
www.roche-applied-science.com/dig

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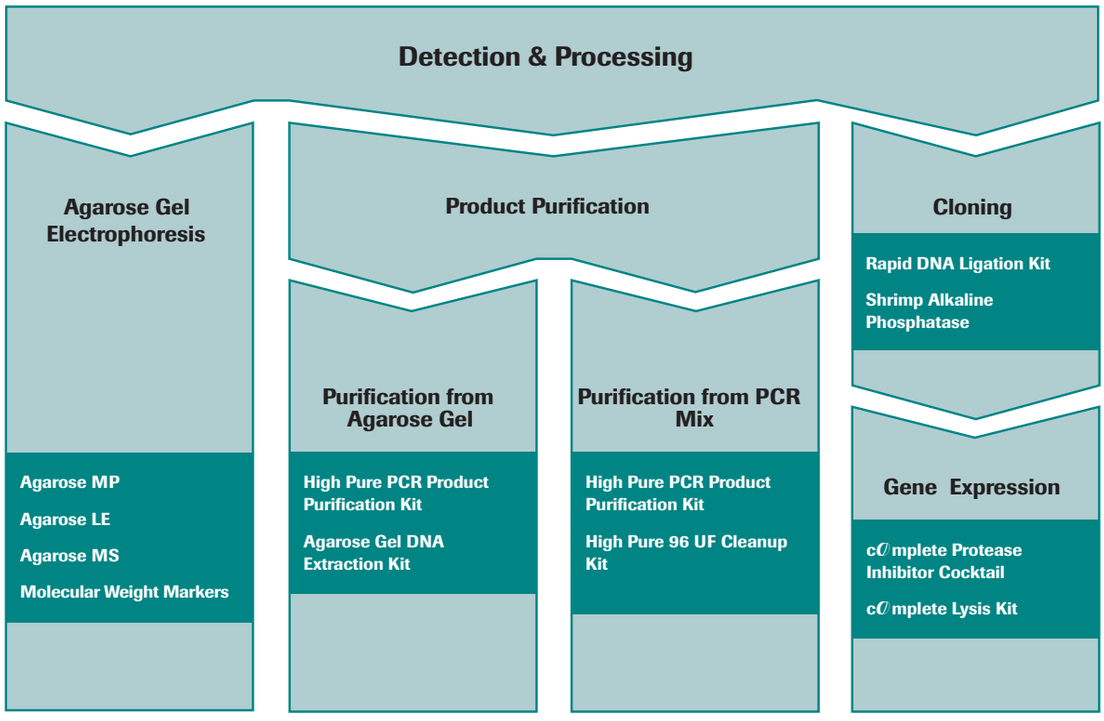
Detection & Processing – Overview

After amplification of the target sequence, the PCR product can be visualized in an agarose gel by electrophoretic separation and staining with a fluorescent dye. For downstream applications like the expression of the gene product, the PCR product has to be cloned into expression vectors. For many applications, the amplification product has to be purified prior to the next step. For convenient and fast results, Roche Applied Science analyzed the activity of restriction enzymes in various PCR buffers, and offers a sophisticated solution for gene expression. Select the reagent that best meets your needs, and be confident you have selected a proven reagent of the highest quality.



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Agarose Gel Electrophoresis

Evaluate your results with confidence

The analysis of a qualitative PCR and RT-PCR amplification reaction is commonly done via agarose gel electrophoresis. The correct length of the amplified fragment is determined by electrophoresis of the products in agarose gels and the visual analysis via comparison with molecular weight markers or other control elements.

Agarose LE

Low electroendosmosis

Choose Agarose LE for analytical and preparative electrophoresis of nucleic acids in routine gel electrophoresis.
The appropriate size range of nucleic-acid separation with Agarose LE lies between 0.2 to 15 kbp, depending on the concentration of agarose applied. Therefore, Agarose LE can be used for the analysis of PCR products, examination of restriction-endonuclease digest of plasmid, cosmid and lambda-phage DNA, and electrophoresis of RNA.

Product	Cat. No.	Pack Size
Agarose LE	11 685 660 001	100 g
	11 685 678 001	500 g

Agarose MS

Molecular screening

Choose Agarose MS for the separation of small DNA fragments (50 – 1500 bp). It is particularly well suited to discriminate between fragments that differ in only four base pairs of length. The high-strength gels are easy to handle and exhibit minimal cracking.
It is ideal for applications such as separation of PCR products, genotyping, allele sizing, and short tandem repeat analysis.

Product	Cat. No.	Pack Size
Agarose MS	11 816 586 001	100 g
	11 816 594 001	500 g

Agarose MP

Multi purpose

Choose Agarose MP for the separation of nucleic acid fragments of all sizes, including the separation of high molecular weight fragments (*e.g.,* chromosomes and fragments of genomic DNA). Agarose MP is specially formulated, and a high gel-strength agarose. The high gel-strength permits the use of low concentrations of agarose in electrophoretic gels and allows rapid migration of high molecular-weight DNA. Furthermore, it reduces separation times for large DNA molecules while maintaining high resolution.

Product	Cat. No.	Pack Size
Agarose MP	11 388 983 001	100 g
	11 388 991 001	500 g

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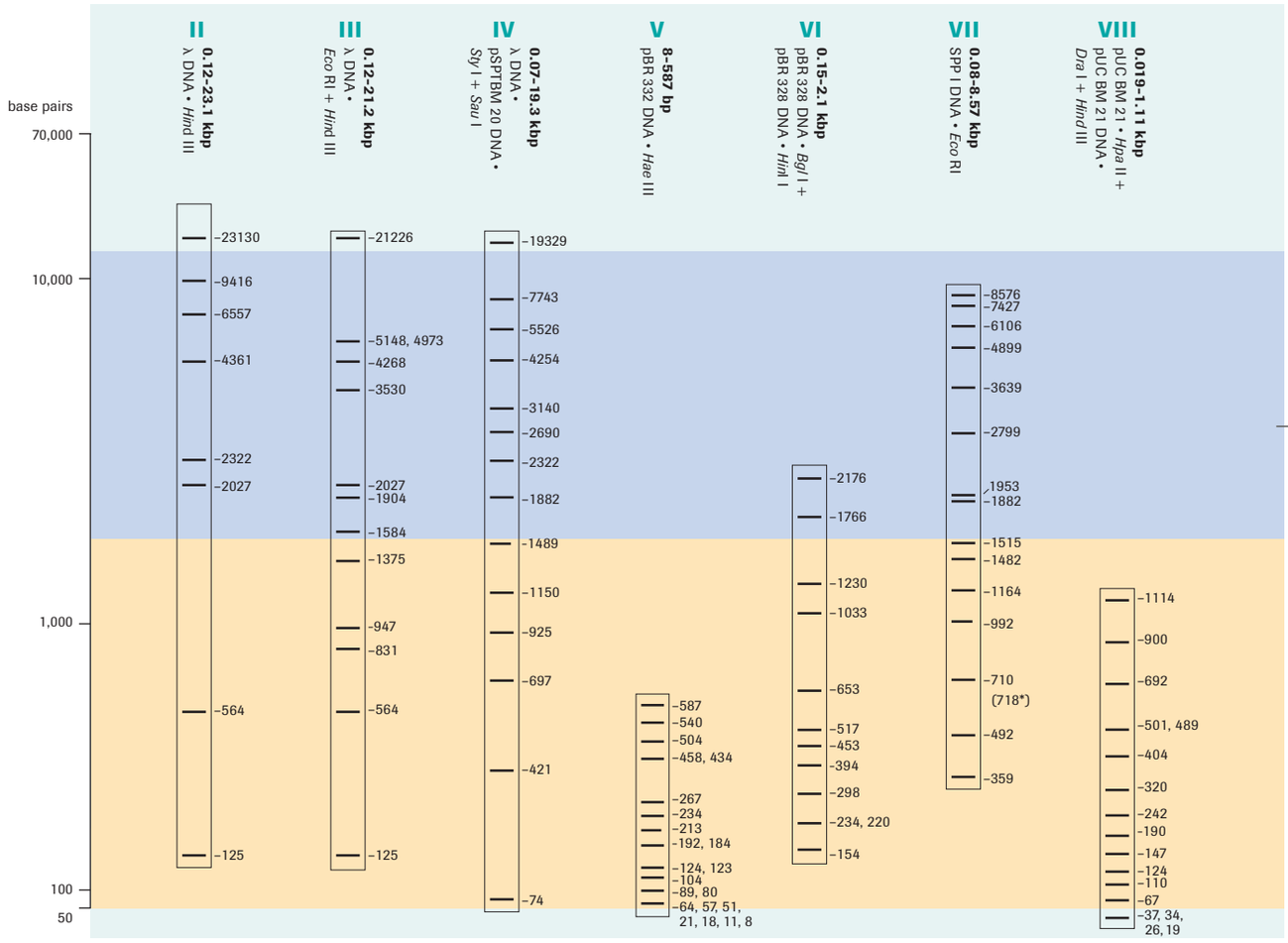
DNA Molecular Weight Markers

Size precise

Rely on Roche Applied Science's DNA Molecular Weight Markers for accurate molecular weight determination on agarose gels of double-stranded DNA fragments from PCR or restriction digests.



Product	Cat. No.	Pack Size
DNA Molecular Weight Marker II ⊙	10 236 250 001	50 µg
DNA Molecular Weight Marker III ⊙	10 528 552 001	50 µg
DNA Molecular Weight Marker IV ⊙	11 418 009 001	50 µg
DNA Molecular Weight Marker V ⊙	10 821 705 001	50 µg

Product	Cat. No.	Pack Size
DNA Molecular Weight Marker VI ⊙	11 062 590 001	50 µg
DNA Molecular Weight Marker VII ⊙	11 209 264 001	50 µg
DNA Molecular Weight Marker VIII ⊙	11 336 045 001	50 µg

⊙ Also available in a DIG-labeled form.

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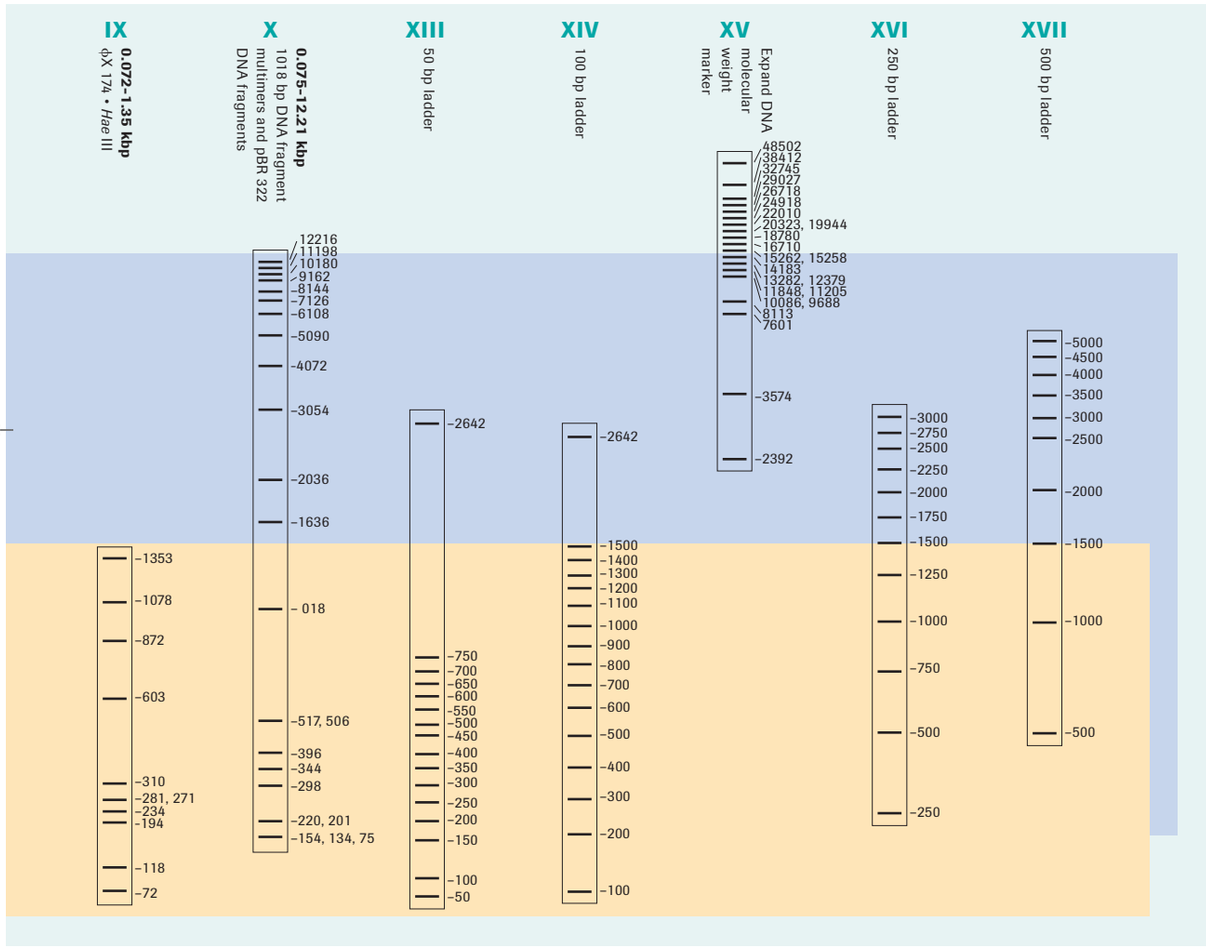
Choose from a wide variety of products the optimal combination of Molecular Weight Marker and Agarose that best fits your needs. Many of our Molecular Weight Markers are also available in a DIG-labeled form (marked with ⊙).



Product	Cat. No.	Pack Size
DNA Molecular Weight Marker IX	11 449 460 001	50 µg
DNA Molecular Weight Marker X	11 498 037 001	100 µg
DNA Molecular Weight Marker XIII	11 721 925 001	50 µg
DNA Molecular Weight Marker XIV	11 721 933 001	50 µg

Agarose MS 0.05 Kbp – 1.5 Kbp Agarose LE 0.2 Kbp – 15 Kbp
Agarose MP 0.1 Kbp – 30 Kbp

Product	Cat. No.	Pack Size
DNA Molecular Weight Marker XV	11 721 615 001	50 µg
DNA Molecular Weight Marker XVI	11 855 638 001	50 µg
DNA Molecular Weight Marker XVII	11 855 646 001	50 µg

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PCR Product Purification

Flexibility unlimited

High Pure PCR Product Purification Kit

Efficiently purify 5 to 25 µg of DNA from PCR, modifying (*e.g.*, alkaline phosphatase), labeling, or restriction digest reactions with the High Pure PCR Product Purification Kit.

- **Insist on high yields.**
  High recovery of starting material by avoiding the substantial DNA loss caused by precipitation-based methods.
- **Save time.**
  Multiple PCR products can be purified in parallel in 10 minutes.
- **Take advantage of a convenient kit.**
  Easy-to-use spin columns including glass fiber fleece eliminate contaminants and short DNA fragments (<100 bp).
- **Elute in low-salt buffer.**
  The purified DNA can be used directly in cloning, sequencing, *etc.*

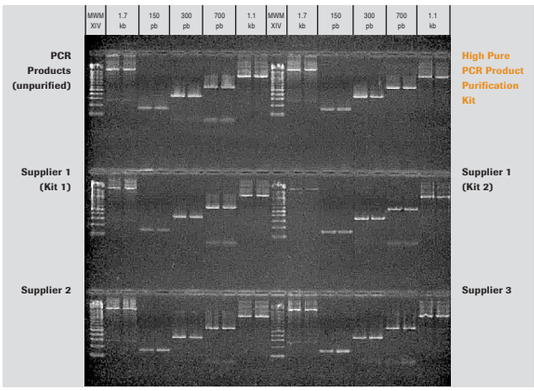

Figure 21: Comparison of PCR product purification with the High Pure PCR Product Purification Kit or other suppliers' kits.
Five PCR products of differing lengths (approximately 150 bp; 300 bp; 700 bp; 1.1 kb; 1.7 kb) were purified using either the Roche Applied Science High Pure PCR Product Purification Kit or products from competitors, and analyzed on a 1.5% agarose gel (Agarose MS). Contaminating primer-dimers were successfully removed with the Roche Applied Science kit, but not by the four competitive products (Supplier 1's Kit 2 nearly failed to purify the 1.7-kb fragment).
Result: High recovery of PCR products and complete removal of low molecular weight material is only obtained with the Roche Applied Science High Pure PCR Product Purification Kit.

Product	Cat. No.	Pack Size
High Pure PCR Product Purification Kit *	11 732 668 001	50 purifications
	11 732 676 001	250 purifications

High Pure 96 UF Cleanup Kit

Choose the High Pure 96 UF Cleanup Kit for efficient and reliable isolation of highly pure and concentrated PCR fragments for high-throughput applications, including fluorescent sequencing, labeling, cloning, restriction digest, and microarray spotting. Simply load, filter, suspend, and recover your purified PCR Product!

- **Choose a flexible format.**
  Samples can be processed manually, or automated with common liquid-handling instruments using vacuum manifolds, or microplate centrifuges.
- **Reduce purification time.**
  All reagents and plates are ready-to-use and supplied with the kit.
- **Benefit from a quick and simple procedure.**
  96 samples can be processed in parallel less than 20 minutes.
- **Persist on reliability.**
  Highly purified DNA, over a broad range of PCR fragment length down to 150 bp, is obtained.
- **Easily recover DNA from the robust ultrafiltration membrane.**
  No interference of membrane parts in microarray spotting.
- **Avoid cross contamination.**
  No well-to-well or aerosol crosstalk due to 96 individuell columns.

Product	Cat. No.	Pack Size
High Pure 96 UF Cleanup Kit *	04 422 694 001	192 purifications

Agarose Gel DNA Extraction Kit

Use this kit to efficiently purify specific DNA fragments from standard or low melting point agarose with excellent yield. It is an ideal tool for concentrating aqueous DNA solutions. The isolated DNA fragments are efficiently ligated into cloning vectors, or labeled to high specific activity through random primed labeling or nick translation. No inhibition of digestion with restriction enzymes is observed.

- **Benefit from a quick and simple procedure.**
  DNA is extracted in only 45 minutes, and with a few hands-on steps.
- **Combine with different agaroses and buffer systems.**
  Low melting point agarose is not required.
- **Purify efficiently.**
  The highly specific binding of DNA allows easy removal of impurities.
- **Insist on a gentle procedure.**
  Shearing of large DNA fragments is prevented due to the uniformity and smooth surface of the silica particles.
- **Avoid enzymatic inhibition.**
  The suspension is free of fines and small particles which saves subsequent reactions.

Product	Cat. No.	Pack Size
Agarose Gel DNA Extraction Kit	11 696 505 001	Up to 100 reactions

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Cloning of PCR Products

A tradition of premium quality

Achieve faster results in various downstream applications (*e.g.*, gene expression, *in vitro* transcription, sequencing, preparation of labeled probes) with Roche Applied Science's optimized solutions for every step in your application. For various reasons, including direct cloning of amplified DNA, researchers want to add restriction enzymes directly to a PCR reaction mix and digest the PCR product without first purifying it. The following restriction enzymes show sufficient activity when used directly in a standard Taq DNA Polymerase or Pwo SuperYield DNA Polymerase reaction buffer.

Product	Relative Activity in			Cat. No.	Pack Size* (units)
	①	②	③		
Alu I	100			10 239 275 001	500
Apa I	100	10	>100	10 899 208 001	5,000
Asp 718	100			10 814 245 001	1,000
*Bam*H I	100	100	>100	10 220 612 001	1,000
*Bbr*P I	100			11 168 860 001	500
Bfr I	100			11 198 939 001	500
Bgl II		85	100	10 348 767 001	500
*Bss*H *II*	100			11 168 851 001	200
*Bst*E II	100			10 404 233 001	500
Cfo I	100			10 688 541 001	1,000
Cla I	100	>100	>100	10 404 217 001	500
Dpn I	100	100	100	10 742 970 001	200
Dra I	100			10 827 754 001	5,000
Hae III	100			10 693 944 001	5,000
*Hin*d II	100			10 656 305 001	2,500
Hpa I	100			10 380 385 001	100
Kpn I		100	100	10 899 186 001	5000
Nco I		100	50	10 835 315 001	200
Nde I		40	100	11 040 219 001	200
Nhe I	100	>100	>100	10 885 843 001	200
Nru I	75	10	25	10 776 769 001	200
Nsi I	100			10 909 831 001	200
Pst I	90	35	15	10 621 625 001	3,000
Pvu II	100			10 642 690 001	1,000
Rsa I	100			10 729 124 001	1,000
Sac I	100	100	20	10 669 792 001	1,000
*Sau*3A I	100			10 709 751 001	500
Sma I	100	>100	100	10 220 566 001	1,000
Taq I	100			10 567 671 001	2,500
Xba I		25	100	10 674 257 001	1000

Table 3: Activity of restriction enzymes in PCR mixes.

Cloning of amplified DNA is often a difficult step in analyzing the products of a polymerase chain reaction. To facilitate cloning of PCR products, several tools have been developed.

*For more information about our products for Mapping & Cloning, including all available pack sizes of Roche Applied Science's restriction enzymes,visit our Special Interest Site **www.restriction-enzymes.com**

① Relative activity (%) in PCR mix including Taq DNA Polymerase, dNTPs, primers and buffer.
② Relative activity (%) in PCR mix including Pwo SuperYield DNA Polymerase, dNTPs, primers and buffer.
③ Relative activity (%) in PCR mix as in ② but additionally supplemented with GC-RICH Solution.

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Rapid DNA Ligation Kit

Employ the Rapid DNA Ligation Kit to enable fast ligation of sticky– or, blunt-end DNA fragments in just 5 minutes at 15°C to 25°C. Depending on DNA concentration, either circular (low DNA concentration) or concatameric (high DNA concentration) ligation products are formed. All necessary reagents required for ligation are provided. There is no need to prepare buffers, or to add ATP or Mg^{2+}.

- **Insist on short ligation times.**
  Only 5 minutes for either blunt- or sticky-ended ligation reaction is needed.
- **Benefit from convenience and ease of use.**
  Ligation reaction is performed at room temperature and all ready-to-use buffers and enzymes are included.
- **Enjoy highest flexibility.**
  The kit is suitable for all common ligation reactions.

Product	Cat. No.	Pack Size
Rapid DNA Ligation Kit	11 635 379 001	40 DNA ligations

Alkaline Phosphatase, shrimp

Use Alkaline Phosphatase, shrimp (SAP) to catalyze the dephosphorylation of 5' phosphates from DNA and RNA. SAP is equally efficient on either 5'-protruding, 5'-recessive and blunt ends. Unlike calf intestinal phosphatase, SAP is completely and irreversibly inactivated by heat treatment for 15 minutes at 65°C. The whole procedure including restriction enzyme digestion, dephosphorylation, enzyme inactivation, and ligation with the Rapid DNA Ligation Kit is performed in one single tube.

Product	Cat. No.	Pack Size
Alkaline Phosphatase, shrimp	11 758 250 001	1,000 units

PCR Cloning Kit

Use the PCR Cloning Kit (blunt end) for a fast, efficient, and convenient method of cloning small DNA fragments (<1.5 kb) as well as larger DNA fragments (up to 10 kb). Blunt-ended DNA fragments (*e.g.*, PCR fragments) can be used directly for rapid ligation (5 minutes) to achieve high cloning efficiencies. Any blunt-ended DNA fragment may be used directly for cloning purposes, without pretreating the ends (*i.e.*, purification or polishing).

Product	Cat. No.	Pack Size
PCR Cloning Kit *	11 939 645 001	1 kit

Expand Cloning Kit

Use the Expand Cloning Kit for a fast and efficient method of cloning large PCR fragments (7 to 36 kb). The kit provides all the required reagents for performing a one-step reaction (when necessary), where the PCR fragments are polished and subsequently phosphorylated. The Expand vectors are supplied linearized and dephosphorylated. The Expand Cloning Kit is optimized for cloning of long PCR fragments (*e.g.*, generated with the Expand Long Template PCR System).

Product	Cat. No.	Pack Size
Expand Cloning Kit *	11 940 392 001	1 kit

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Gene Expression

Keep it easy

cOmplete Protease Inhibitor Cocktail Tablets in EASYpacks

Rely on the protection you've always obtained with cOmplete Tablets – now in even more convenient packaging. Save time, expense, and handling steps while increasing convenience and avoiding stress.

- **Simplify protection.**
  Proteins are protected against a multitude of proteases by simply adding a cOmplete Tablet to aqueous protein isolation buffer.
- **Use a convenient tablet.**
  Tablets combining different protease inhibitors, with or without EDTA, are available for either 10 ml or 50 ml of lysate.
- **Eliminate weighing small amounts.**
  Sufficient amounts of various inhibitors are conveniently supplied in a tablet form.
- **Insist on a versatile product.**
  Proteases are inhibited in extracts from almost any tissue or cell type, including animals, plants, yeast, bacteria, or fungi.

Product	Cat. No.	Pack Size
cOmplete	04 693 116 001	20 tablets (for 50 ml each)
cOmplete, Mini	04 693 124 001	30 tablets (for 10 ml each)
cOmplete, EDTA-free	04 693 132 001	20 tablets (for 50 ml each)
cOmplete, Mini, EDTA-free	04 693 159 001	30 tablets (for 10 ml each)

cOmplete Lysis Kits

Obtain effective, easy cell lysis and convenient, reliable protease inhibition in the same package by choosing one of our new cOmplete Lysis products.

- **Perform gentle cell lysis in 5–20 minutes*.**
  Traditional lysis methods such as freeze-thaw cycles, sonication, or glass beads are not needed.
- **Obtain high yields of extracted protein.**
  The reagents of the cOmplete Lysis kits reliably inhibit proteases and do not denature or interact with proteins.

* Lysis requires 5 minutes for mammalian cells, 10 minutes for bacteria, and 20 minutes for yeast cells.

Cell Type	Product	Cat. No.	Pack Size
Bacteria	cOmplete Lysis-B (2x)	04 719 930 001	1 kit (100 ml (2x) lysis reagent/ 20 tablets)
	cOmplete Lysis-B (2x), EDTA-free	04 719 948 001	1 kit (100 ml (2x) lysis reagent/ 20 tablets)
Mammalian cells	cOmplete Lysis-M	04 719 956 001	1 kit (200 ml (2x) lysis reagent/ 20 tablets)
	cOmplete Lysis-M, EDTA-free	04 719 964 001	1 kit (200 ml lysis reagent/ 20 tablets)
Yeast cells	cOmplete Lysis-Y	04 719 972 001	1 kit (200 ml lysis reagent/ 20 tablets)
	cOmplete Lysis-Y, EDTA-free	04 719 999 001	1 kit (200 ml lysis reagent/ 20 tablets)

For more information, visit
www.keep-it-easy.com

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Resources

The information provider for life science

By exploiting our 50-years of experience in developing biochemicals and enzymes, Roche Applied Science (RAS) invented and perfected a broad range of new technologies and powerful enzyme preparations for PCR. RAS also pioneered enzyme blending, and was the first company to introduce the enzyme blends Expand High Fidelity and Long Template.
We believe the full potential of the PCR technology is yet to be exploited. Therefore, we will continue serving the scientific community by providing innovative enzymes, reagents, and services, reflecting our philosophy that innovation and quality are the driving factors for your success.

Use the following services and tools supplied by Roche Applied Science to support you in your daily research needs:

Access detailed information about all our products related to PCR and RT-PCR by visiting our PCR Special Interest Site:
www.roche-applied-science.com/pcr

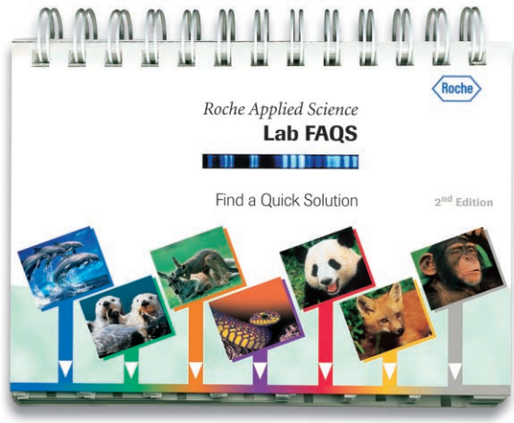
Roche Applied Science introduced the first restriction enzymes in 1976. Since then, many researchers have chosen to apply our enzymes in their everyday work and have relied on the quality and consistency we provide. Learn more about our solutions for mapping & cloning by visiting our Special Interest Site: **www.restriction-enzymes.com**

Achieve immediate assistance for your research application by contacting our Online Technical Support (OTS) at:
www.roche-applied-science.com/ots

The PCR Applications Manual provides PCR techniques, tips, and problem-solving strategies, a convenient overview of many current PCR applications and a description of our line of PCR "tools". This manual is also available online:
www.roche-applied science.com/prod_inf/manuals/pcr_man/start.html

Calculate the annealing temperature, molecular weight and absorbance factor of a given oligonucleotide up to 60 bases in length with this easy-to-use online tool Benchmate Tm Calculator at:
www.roche-applied-science.com/benchmate
Designed to instantly calculate the melting temperature. Three different formulas can be used to calculate the melting temperature of a given oligonucleotide.
- based on the arbitrary method
- nearest neighbor method
- long probe mathematical method

Use LAB FAQs which offers you concrete, concise answers to most frequently asked questions in modern life science research, in a practical pocket format. If you missed to receive your hard copy find the answer online at:
www.roche-applied-science.com/labfaqs



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Related Products

Kits and Reagents for PCR		
Product	**Cat. No.**	**Pack Size**
DOP PCR Master ▼, ●	11 644 963 001	30 reactions
PCR DIG Probe Synthesis Kit	11 636 090 001	25 reactions
T4 Gene 32 Protein *	10 972 983 001 10 972 991 001	100 µg 500 µg
Tth Pyrophosphatase, thermostable *	11 721 992 001	100 units
PCR Buffer Set	11 699 121 001	2 x 2 ml
PCR Buffer without $MgCl_2$, 10x conc.	11 699 105 001	3 x 1 ml
$MgCl_2$ Stock Solution	11 699 113 001	3 x 1 ml

Companion products		
Product	**Cat. No.**	**Pack Size**
COT Human DNA	11581074001	500 µg (500 µl)
Human t-PA Control Primer Set *	11691104001	1 set
DNA, lambda	10745782001	1 ml (5 A_{260} units)
Glycogen	10901393001	20 mg (1 ml)
Primer "random"	11034731001	2 mg
Primer for cDNA Synthesis $p(dT)_{10}$	10814261001	40 µg
Primer for cDNA Synthesis $p(dT)_{15}$	10814270001	40 µg
Streptavidin-coated PCR Tubes (Strips) *	11741772001	24 strips (0.2 ml)
Strip PCR Tubes and Caps *	11 667 009 001 11 667 017 001	125 strips (8 tubes/strip) 80 strips (12 tubes/strip)
Water, PCR-Grade	03 315 932 001 03 315 959 001 03 315 843 001	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)

Real-Time PCR Reagents		
Product	**Cat. No.**	**Pack Size**
FastStart TaqMan® Probe Master (Rox) ¥	04 673 450 001 04 673 468 001 04 673 476 001	2.5 ml (2 x 1.25 ml) 12.5 ml (10 x 1.25 ml) 50 ml (10 x 5 ml)
FastStart TaqMan® Probe Master ¥	04 673 409 001 04 673 417 001 04 673 433 001	2.5 ml (2 x 1.25 ml) 12.5 ml (10 x 1.25 ml) 50 ml (10 x 5 ml)
FastStart SYBR Green Master (Rox) ◖, ✦	04 673 514 001 04 673 522 001	5 ml (4 x 1.25 ml) 50 ml (10 x 5 ml)
FastStart SYBR Green Master ◖, ✦	04 673 484 001 04 673 492 001	5 ml (4 x 1.25 ml) 50 ml (10 x 5 ml)
ROX Reference Dye *	04 673 549 001	50 µl

Choose the powerful combination of the ProbeFinder Software, the Universal ProbeLibrary, the Transcriptor First Strand cDNA Synthesis Kit, and the FastStart TaqMan® Probe Master to revolutionize the way you design and perform real-time qPCR assays. For more information, please refer to **www.universalprobelibrary.com**

For more information about reagents for the LightCycler® Instruments, please refer to the LightCycler® Instruments Special Interest Site at **www.lightcycler.com**

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‡ US patent granted (6.3.99.320), corresponding patent applications in Europe, Canada, Japan and Russia are pending.

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