

High Pure PCR Product Purification Kit

for purification of DNA from PCR reactions

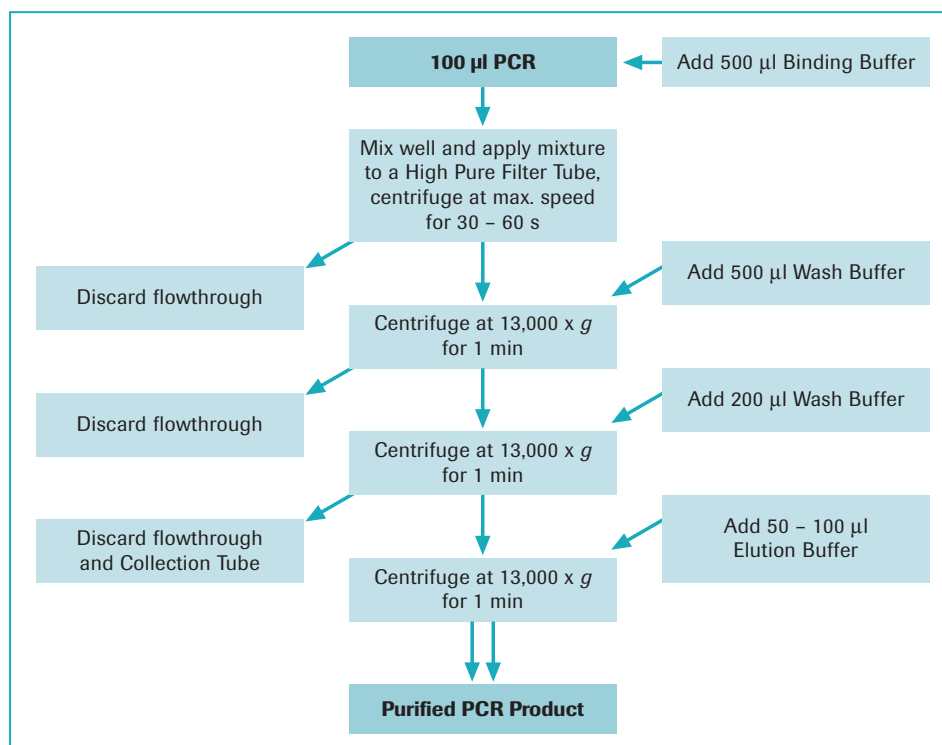
Cat. No. 11 732 668 001 (up to 50 purifications)

Cat. No. 11 732 676 001 (up to 250 purifications)

Principle	In the presence of chaotropic salt, product DNA binds selectively to glass fiber fleece in a special centrifuge tube. The DNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating small molecules (including small nucleic acids). Finally, low salt elution removes the DNA from the glass fiber fleece. The process does not require DNA precipitation, organic solvent extractions, or extensive handling.
Starting material	<ul style="list-style-type: none"> ● Research samples (100 µl) may be: ● DNA products (>100 bp – 50 kb) from PCR ● Enzymatically labeled, modified, or digested DNA (for example, products from restriction digests, alkaline phosphatase treatments, kinase reactions, or enzymatic labeling reactions) ● RNA from <i>in vitro</i> transcription reactions ● DNA from a 100 mg agarose gel slice
Application	<ul style="list-style-type: none"> ● Preparation of concentrated, purified DNA, which may be used directly for labeling, sequencing, cloning and other routine applications
Time required	<ul style="list-style-type: none"> ● Total time: approx. 10 min ● Hands-on time: <10 min
Results	<ul style="list-style-type: none"> ● Yield: Variable, depending on sample volume and DNA size (See the table under Part IV of “How to use the kit” in this article) ● Purity: Purified DNA is free of short DNA (<100 bp), small molecules (for example, mineral oil, primers, salts, unincorporated nucleotides) and proteins (for example, thermostable enzymes)
Benefits	<ul style="list-style-type: none"> ● Saves time, because the kit makes PCR products ready for downstream procedures in less than 10 min ● Minimizes DNA loss, because the kit removes contaminants without precipitation or other handling steps that lead to DNA loss or degradation ● Increases lab safety, because the kit does not use hazardous organic solvents ● Accommodates a wide variety of samples, because the kit can purify DNA from most enzymatic reaction mixtures, DNA from agarose gels, even RNA from <i>in vitro</i> transcription reactions ● Improves performance of downstream applications, because the kit removes DNA fragments smaller than 100 bp from the preparation

How to use the kit

I. Flow diagram



II. Kit contents

- Binding Buffer with guanidine thiocyanate (30 ml or 150 ml)
- Wash Buffer (10 ml or 50 ml)
 - ⚠ Add absolute ethanol (40 ml or 200 ml) to Wash Buffer before first use
- Elution Buffer (Tris-HCl buffer, pH 8.5) (40 ml)
- High Pure Filter Tubes (50 or 250 tubes)
- Collection Tubes, 2 ml (50 or 250 tubes)
 - ⚠ Both sizes of the kit contain the same components; only the amount of the components in the kit changes.

III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Agarose
- TAE Buffer (40 mM Tris-acetate, 1 mM EDTA), pH 8.0
- TBE Buffer (89 mM Tris-borate, 2 mM EDTA) pH 7.8
- Electrophoresis equipment
- Sterile scalpel
- Isopropanol

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IV. Expected DNA recovery from various amounts and sizes of DNA

DNA applied (µg)	Recovery (%)	Fragment ^{1,2} length (bp)	Recovery (%)	Elution volume ¹ (µl)	Recovery (%)
5	77	<100	< 5	50	68
10	79	375	>95	100	79
25	80	700	>95	150	80
50	56	3000	>95	200	80

¹ 10 µg DNA was used for this experiment.

² 200 µl Elution Buffer was used for this experiment.

V. Protocols for preparing DNA


Va. Purifying amplification products from 100 µl PCR product mix





To process a larger sample (>100 µl), either increase proportionally the amount of Binding Buffer (Step 1) or divide the large sample into several aliquots and process the aliquots as separate samples.

- 1 After the PCR amplification is complete, adjust the volume in one PCR tube (reaction components + DNA product) to 100 µl, then:
 - ▶ Add 500 µl Binding Buffer to the contents (100 µl) of the PCR tube.
 - ! You do not need to remove mineral oil or wax from the PCR solution before adding the Binding Buffer.
 - ▶ Mix the sample (Binding Buffer + starting PCR solution) well.
- 2 After mixing the sample:
 - ▶ Insert one High Pure Filter Tube into one Collection Tube.
 - ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube.
- 3 Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 30 s at full speed (approx. 13,000 x g).
- 4 After the centrifugation:
 - ▶ Remove the Filter Tube from the Collection Tube.
 - ▶ Discard the flowthrough liquid in the Collection Tube.
 - ▶ Reinsert the Filter Tube in the same Collection Tube.
- 5 To wash the sample:
 - ▶ Add 500 µl Wash Buffer to the upper reservoir of the Filter Tube.
 - ▶ Repeat the centrifugation (as in Step 3).
- 6 After the first wash:
 - ▶ Discard the flowthrough and recombine the tubes (as in Step 4).
 - ▶ Add 200 µl Wash Buffer to the upper reservoir of the Filter Tube.
 - ▶ Repeat the centrifugation (as in Step 3).



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- 7 After the second wash:
- ▶ Remove the Filter Tube from the Collection Tube.
 - ▶ Discard the Collection Tube (with the flowthrough liquid).
 - ▶ Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
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- 8 To elute the DNA from the Filter Tube:
- ▶ Add 50 – 100 µl Elution Buffer or double dist. water (pH 8.0 – 8.5) to the upper reservoir of the Filter Tube.
 - ▶  *For optimal recovery of DNA, use 100 µl buffer or water.*
 - ▶ Centrifuge the tube assembly for 30 s at full speed.
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- 9 The microcentrifuge tube now contains the eluted DNA. You may:
- ▶ EITHER use the eluted DNA directly in such applications as cloning or sequencing
 - ▶ OR store the eluted DNA at 2 to 8°C for later analysis.
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Vb. Purifying DNA from a 100 mg slice of agarose gel

- 1 Purify the DNA of interest electrophoretically as follows:
- ▶ Prepare an agarose gel with Roche Applied Science agarose (MP, LE or MS) and load the DNA of interest on the gel.
 - ▶ Use 1 x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) or 1 x TBE (45 mM Tris-borate, 1 mM EDTA, pH 8.0) as running buffer.
 - ▶ Electrophorese until the DNA of interest is well separated from contaminants.
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- 2 After gel electrophoresis, stain the gel with ethidium bromide.
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- 3 Cut the DNA band from the gel with a sharp scalpel or razor blade.
-  *Cut the smallest possible gel slice.*
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- 4 Place the agarose gel slice into a sterile 1.5 ml microcentrifuge tube of known weight, then reweigh the tube to determine the weight of the agarose gel.
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- 5 To the microcentrifuge tube, add 300 µl Binding Buffer for every 100 mg agarose gel.
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- 6 To dissolve the agarose gel slice and release the DNA:
- ▶ Vortex the microcentrifuge tube briefly to resuspend the gel slice in the Binding Buffer.
 - ▶ Incubate the suspension for 10 min at 56°C.
 - ▶ Vortex the tube briefly every 2 – 3 min during the incubation.
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- 7 After the gel is completely dissolved:
- ▶ To the microcentrifuge tube, add 150 µl isopropanol for every 100 mg agarose gel.
 - ▶ Vortex thoroughly.
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- 8 After mixing the components of the microcentrifuge tube:
- ▶ Insert one High Pure Filter Tube into one Collection Tube.
 - ▶ Pipette the entire contents of the microcentrifuge tube into the upper reservoir of the Filter Tube.
-  *If the volume of gel suspension is >700 µl, divide the suspension into two portions and use separate Filter Tubes for each portion.*
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- 9 Follow Protocol Va above, starting at the centrifugation step (Step 3).
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Vc. Purifying labeled probes

1 Fill up the labeling reaction to 100 µl with double dist. water.

2 Add 500 µl Binding Buffer and mix well.



It is important that the volume ratio between sample and Binding Buffer is 1:5. When using other sample volumes than 100 µl, adjust the volume of Binding Buffer accordingly.

3 Follow Protocol Va starting at the sample transfer to the Filter Tube assembly (Step 2).



The elution efficiency is increased with higher volume of Elution Buffer applied. At least 68% and 79% recovery are found with 50 and 100 µl Elution Buffer, respectively. Normally, almost quantitative recovery can be found, as can be determined in a direct detection assay.

VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure PCR Product Purification Kit, see page 101.

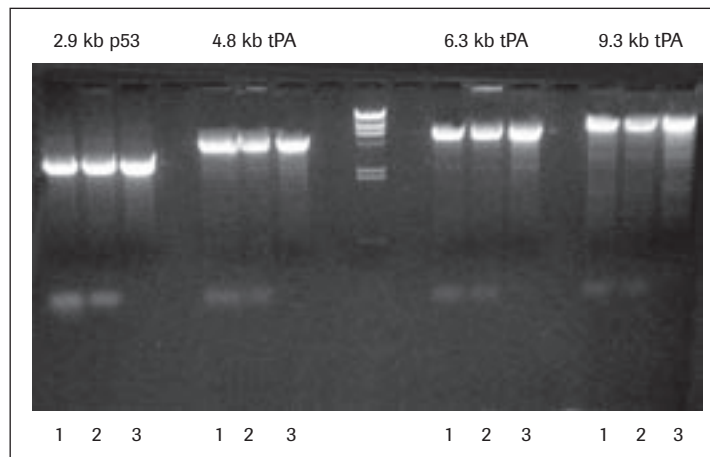
Typical results with the kit**Experiment 1**

Figure 8: Comparison of PCR products purified with the High Pure PCR Product Purification Kit and those purified by phenol/chloroform extraction.

Four different long PCR products were generated with the Expand Long Template PCR System, purified by either of two methods, then analyzed electrophoretically. From left to right, the four products shown on the gel are: a 2.9 kb fragment from the p53 gene; 4.8 kb, 6.3 kb, and 9.3 kb fragments from the tissue plasminogen activator (tPA) gene. For each size product, the gel shows:

Lane 1: DNA before purification

Lane 2: DNA after purification by phenol/chloroform extraction

Lane 3: DNA after purification with the High Pure PCR Product Purification Kit

Result: The High Pure Kit removed primers and primer dimers, while recovering 90% of the long PCR products (as calculated by photospectroscopy).

Experiment 2

Preparation of hybridization probes for the analysis of number and expression patterns from sulfurtransferases from *Arabidopsis*

(kindly provided by J. Papenbrock, P. von Trzebiatowski and A. Schmidt, University of Hannover, Germany)

Background: Sulfurtransferases are a group of enzymes widely distributed in plants, animals, and bacteria. They catalyze the transfer of a sulfane atom from a donor molecule to a thiophilic acceptor substrate.

In a database search several *Arabidopsis* EST clones with homologies to bacterial and mammalian sulfurtransferases could be identified. One of them was used to isolate a full length clone by screening an *Arabidopsis* cDNA library. The protein was expressed in *E. coli* and was determined to be a sulfurtransferase with high specificity for 3-mercaptopyruvate. However, RNA expression data, protein contents obtained by Western blot analysis and sulfurtransferase enzyme activity measurements using 3-mercaptopyruvate or thiosulfate as substrates did not correlate with each other in *Arabidopsis* plants in different developmental states. Therefore, we hypothesize the existence of further sulfurtransferases in *Arabidopsis*. Different EST clones that show homology to sulfurtransferases and also to senescence-associated proteins are initially characterized.

Methods: Genomic Southern blot was performed as previously described. For preparing the hybridization probes cDNAs of interest were separated from the vectors with the respective restriction enzymes (MST1 coding for the mature 3-mercaptopyruvate sulfurtransferase, 950 bp, *Kpn* I/*Pst* I; EST 46D8T7, 600 bp, *Sal* I/*Not* I). DNA fragments were separated by agarose gel electrophoresis. The gel pieces containing the MST1 or the 46D8T7 fragment were eluted following exactly the inserted recipe for isolating DNA from agarose gels using the High Pure PCR Product Purification Kit. Eluted DNA was used for random prime labeling.

Northern blot was performed as previously described. The PCR probe used in Northern blotting was purified in the following way: The gel piece of interest was excised and the labeled fragment was purified with the High Pure PCR Product Purification Kit according to the inserted recipe for isolating DNA from agarose gels with an elution volume of 100 μ l.

Results: Hybridization of restricted genomic *Arabidopsis* DNA with a digoxigenin-labeled MST1 probe by random priming reveals the existence of at least two probably three different sulfurtransferase genes in the *Arabidopsis* genome (Figure 9, right). The pattern is similar to the hybridization results using the 46D8T7 cDNA but not identical (Figure 9, left).

RNA homologous to the sequence of EST clone 46D8T7 was expressed in very young plants. The expression level decreases drastically and increases continuously during aging (Figure 10). In the plants of a later developmental stage, expression level parallels to the expression of SAG 13 clone, the best marker of senescence known so far.

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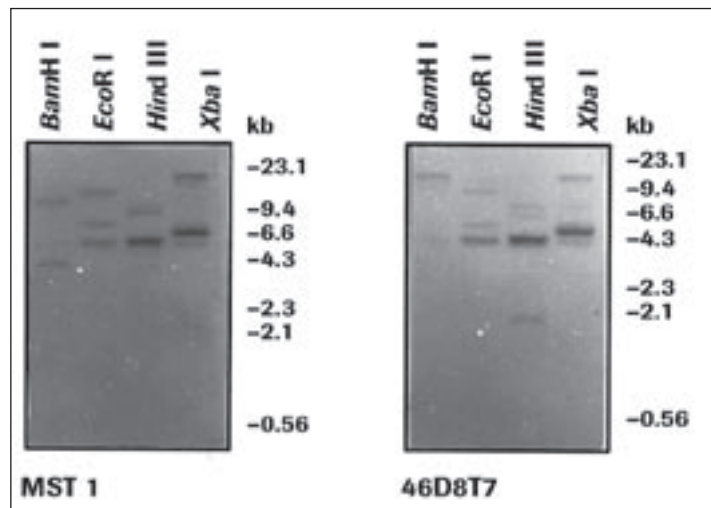


Figure 9: Southern blot analysis. Genomic DNA was restricted with BamH I, EcoR I, Hind III, and Xba I overnight, transferred to nylon membrane and hybridized with a digoxigenin probe labeled by random prime labeling.

Left: MST1 (3-mercapto-pyruvate sulfurtransferase) cDNA was used as a probe.

Right: cDNA from the EST clone 46D8T7 was labeled.



Figure 10: Northern blot analysis. Total RNA was extracted from Arabidopsis plants of different ages (from the left to the right: harvested after 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 days), separated under denaturing conditions and transferred to nylon membranes. Equal loading was controlled by ethidium bromide staining. Filters were hybridized with PCR fragments labeled with digoxigenin. PCR-probe (600 bp) used for hybridizing the filter at the top: cDNA from EST clone 46D8T7. PCR-probe (1300 bp) used for hybridizing the filter at the bottom: cDNA from the SAG13 clone (senescence associated gene).

Discussion: The use of digoxigenin-labeled probes leads to very sharp bands in genomic Southern blotting. Purification of digoxigenin-labeled DNA via PCR using the High Pure PCR Product Purification Kit seems to increase the sensitivity of probes and reduce unspecific binding.

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