

Select the Optimal Enzyme for RT-PCR

A major factor to consider in reverse transcription polymerase chain reaction (RT-PCR) is the choice of the appropriate reverse transcriptase. Roche Applied Science has developed and optimized a wide variety of different enzymes and enzyme blends. Each of these products has different enzymatic properties and one may be more suitable for a specific experiment than the other. The

information given below outlines the individual properties of each enzyme and may help you determine the optimal enzyme or enzyme blend for your specific application.

For more information, visit and bookmark our Amplification Special-Interest Site at www.roche-applied-science.com/pcr or contact your local representative. ■

One-step RT-PCR offers faster reactions, reduced risk of contamination, and improved sensitivity				
	Tth DNA Polymerase	<i>C. therm.</i> Polymerase One-Step RT-PCR System	Titan One Tube RT-PCR System	Titan One Tube RT-PCR Kit
Enzyme component	Thermostable DNA polymerase with intrinsic reverse transcriptase activity	Blend of <i>C. therm.</i> DNA Polymerase and Taq DNA Polymerase	Blend of AMV Reverse Transcriptase and Expand High Fidelity Enzyme Blend	
Product size	Up to 1 kb	Up to 3 kb	Up to 6 kb	
Priming	Specific	Specific	Specific	
Reaction temperature	55°C – 70°C	60°C – 70°C	45°C – 60°C	
RNase H activity	–	–	++	
Ion requirement	Mn ²⁺	Mg ²⁺	Mg ²⁺	
Sensitivity – total RNA	+	++	+++	
Sensitivity – mRNA	+	++	+++	
Sensitivity – viral RNA	+	+++	+	
Sensitivity – RNA rich in secondary structures	+	+++	+	
Specificity	+	++	+	
Fidelity	+	++	+++	
Full-length cDNA	–	+	++	
Incorporation of modified nucleotides	Yes	Yes	Yes	
Carry-over prevention (dUTP/UNG)	Yes	Yes (first choice for fragments up to 3 kb)	No	
Buffer system	Both a PCR and RT-PCR buffer are included.	A single reaction buffer is included. Separate vials of DMSO and DTT for optimization of reaction conditions are also included.	A single reaction buffer is included.	
Advantages	<ul style="list-style-type: none"> Resistant to prolonged incubation at high temperatures (95°C) No RNase H activity Carry-over prevention 	<ul style="list-style-type: none"> Improved yield and specificity with GC-rich RNA or RNA forming secondary structures Higher fidelity Longer RT-PCR products No RNase H activity Carry-over prevention 	<ul style="list-style-type: none"> The longest RT-PCR products; up to 6 kb Full-length cDNA Highest sensitivity for mRNA Best fidelity 	
Product contents	<ul style="list-style-type: none"> Enzyme PCR buffer with MgCl₂ RT-PCR buffer Mn(OAc)₂ solution 	<ul style="list-style-type: none"> Enzyme blend RT-PCR buffer with MgCl₂ and DMSO DMSO solution DTT solution 	<ul style="list-style-type: none"> Enzyme blend RT-PCR buffer with MgCl₂ and DMSO MgCl₂ solution DTT solution 	<ul style="list-style-type: none"> Enzyme blend RT-PCR buffer with MgCl₂ and DMSO MgCl₂ solution DTT solution dNTP mix RNase inhibitor human control RNA control primer mix PCR-grade water
Catalog number	1 480 014 (100 units) 1 480 022 (500 units)	2 016 338 (50 reactions) 2 016 346 (250 reactions)	1 888 382 (25 reactions) 1 855 476 (100 reactions)	1 939 823 (50 reactions)

Two-step RT-PCR offers maximum flexibility with respect to primer choice, enzyme, temperature and multiple transcripts analysis

	<i>C. therm.</i> Polymerase	M-MuLV Reverse Transcriptase	Expand Reverse Transcriptase*	Reverse Transcriptase AMV	1st strand cDNA Synthesis Kit
Product size	4 kb	10 kb	14 kb	12 kb	
Priming	Specific, oligo dT	Specific, oligo dT, random hexamer	Specific, oligo dT, random hexamer	Specific, oligo dT, random hexamer	
Reaction temperature	55 °C - 70 °C	37 °C	42 °C (up to 50 °C)	42 °C (up to 60 °C)	
RNase H activity	-	+	-	++	
Ion requirement	Mg ²⁺	Mg ²⁺	Mg ²⁺	Mg ²⁺	
Sensitivity – total RNA	+	+	++	++	
Sensitivity – mRNA	+	+	++	++	
Sensitivity – viral RNA	+++	+	+	+	
Sensitivity – RNA rich in secondary structures	+++	+(+)	+	+	
Specificity	++	not determined	+	+	
Fidelity	++	+	+	+	
Full length cDNA	+	+	+++	++	
Incorporation of modified nucleotides	Yes	Yes	Yes	Yes	
Carry-over prevention (dUTP/UNG)	Yes	not determined	not determined	not determined	
Buffer system	Single reaction buffer is included. Separate vials of DMSO, DTT and Betain for optimization of reaction conditions are also included.	A single reaction buffer is included.	A single reaction buffer is included. A separate vial of DTT for optimization of reaction conditions is also included.	All necessary reagents are included.	
Advantages	<ul style="list-style-type: none"> • High sensitivity with viral and difficult (GC rich) templates • No RNase H activity • Carry-over prevention 	<ul style="list-style-type: none"> • No endo-nuclease activity • Lower RNase H activity than AMV 	<ul style="list-style-type: none"> • cDNA fragments up to 14 kb • full-length cDNA fragments • No RNase H activity 	<ul style="list-style-type: none"> • cDNA fragments up to 12 kb • Higher thermostability and specificity than M-MuLV 	
Product contents	<ul style="list-style-type: none"> • Enzyme • RT-PCR buffer with MgCl₂ and DMSO • DTT solution • DMSO solution • Betain solution 	<ul style="list-style-type: none"> • Enzyme • cDNA synthesis buffer 	<ul style="list-style-type: none"> • Enzyme • RT-PCR buffer • DTT solution 	<ul style="list-style-type: none"> • Enzyme • cDNA synthesis buffer 	<ul style="list-style-type: none"> • Reaction buffer • MgCl₂ • Deoxynucleotide mix • Gelatin • Oligo-p(dT)₁₅ Primer • Random primer p(dN)₆ • RNase inhibitor • AMV Reverse Transcriptase • Control Neo RNA • PCR-grade water
Catalog number	2 016 311 (300 units)	1 062 603 (500 units)	1 785 826 (1,000 units) 1 785 834 (5,000 units)	1 495 062 (500 units) 109 118 (1,000 units)	1 483 188 (1 kit for 30 reactions)

* not for sale in the U.S.