Expand Reverse Transcriptase

from *Escherichia coli*: AP401 (k)

Deoxynucleoside-triphosphate:DNA deoxynucleotidyl-transferase (RNA directed)
E.C.2.7.7.49

Cat. No. 11 785 826 001 1 000 U
Cat. No. 11 785 834 001 5 000 U

Version 13
Content version: March 2020

Store at −15 to −25°C

Product overview

Pack content

Vial	Content
Expand Reverse Transcriptase	▪ 1000 U (Cat. No. 11 785 826 001) ▪ 5000 U (Cat. No. 11 785 834 001)
Expand Reverse Transcriptase Buffer, 5 × conc.	cDNA synthesis buffer (first-strand) 250 mM Tris-HCl, 200 mM KCl, 25 mM $MgCl_2$, 2.5% Tween[2)] 20 (v/v), pH 8.3 (25°C).
Dithiothreitol (DTT) solution	100 mM

Storage and stability
The undiluted enzyme is stable at –15 to –25°C through the expiration date printed on the label. The product is shipped on dry ice.

Storage buffer
50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 10 mM dithioerythritol, 0.05% polydocanol (v/v), 50% glycerol (v/v), pH 8.4 (4°C).

Volume activity
50×10^3 U/ml. One unit is the enzyme activity which incorporates 1.0 nmol TMP into acid insoluble products in 10 min at 37°C with poly (A) × $(dT)_{15}$ as substrate (1).

Description
Expand reverse transcriptase is a RNA directed DNA polymerase. The enzyme is a genetically engineered version of the Moloney Murine Leucaemia Virus reverse transcriptase (M-MuLV-RT). Point mutation within the RNase H sequence reduces the RNase H activity under the detectable level. Therefore decisive improvements are obtained regarding higher amounts of full-length cDNA transcripts and in obtaining longer transcripts in contrast to the native M-MuLV-RT.

Application
The enzyme synthesizes with mRNA or single stranded DNA as substrate, in the presence of a primer, a complementary DNA strand.

There are three approaches to priming reverse transcription:

1. Oligo $(dT)_{12-18}$ binds to endogenous poly $(A)^+$ tail at the 3´end of mammalian mRNA.

2. Random hexanucleotides $(N)_6$ can bind to mRNA templates at any complementary site.

3. Specific oligonucleotide sequences can be used to selectively prime the mRNA of interest.

Oligo $(dT)_{12-18}$ nucleotides are most frequently used to initiate first strand cDNA synthesis, if full-length cDNA synthesis is required. Synthesis of non-full-length cDNAs can be achieved by either employing a specific primer or random hexamer nucleotides.

Main application of the Expand reverse transcriptase is the construction of libraries (2), the RT-PCR and application in the 5´/3´RACE technology.

cDNA transcripts are necessary in the analysis of the structure and expression of prokaryotic and eucaryotic genes. By comparison of cDNA with genomic DNA sequences it is possible to detect intervening sequences, and to analyze the splicing and the genomic recombination events in eucaryotic genes.

General handling instructions

RNA preparation
For high quality eucaryotic mRNA preparations it is necessary to minimize the activity of RNases liberated during cell lysis by using inhibitors of RNases or methods that disrupt cells and inactivate RNases simultaneously. A good overview of the methods is given in (4) as well as in (5). Suitable reagents for the isolation of total RNA/or mRNA are the mRNA Isolation Kit and TriPure Isolation Reagent.

Consequently, it is also important to avoid the accidental introduction of trace amounts of RNases from other potential sources in the laboratory, like glassware, plasticware, contaminating solutions and contamination of the investigators hands.

A number of precautions that can be used to avoid problems with RNases contamination are listed in (4).

In a typical mammalian cell only 1–5% of the total cellular RNA is mRNA. However, most eucaryotic mRNAs carry at their 3' termini a tract of polyadenylic acid residues that is generally long enough to allow mRNAs to be purified by affinity chromatography on oligo(dT)-cellulose or with oligo$(dT)_{20}$ probe, biotin-labeled with streptavidin magnetic particles*.

RT-PCR
Up-and downstream PCR-primers are usually selected from two exons spanning an intron. This allows the detection of DNA contamination of the RNA preparation because the products will differ in size.

If this is not possible, it will always be necessary to treat the RNA preparation with DNase I, recombinant RNase-free prior to RT-PCR.

The amount of cDNA used in RT-PCR reaction may vary depending on the nature of the RNA template.

For the different $MgCl_2$ concentrations to be used in Long Range RT-PCR, see Long Range RT-PCR with the Expand Long Template PCR System*.

Integrity of mRNA
Integrity of mRNA is especially important when cDNA libraries are constructed or 5' ends of mRNAs are investigated. The size of the mRNA can be determined by gel electrophoresis and ethidium bromide staining (4).

The mRNA should appear as a smear between approximately 500 bp and 8 kb. The bulk of the mRNA should be between 1.5 and 2 kb.

Priming reverse transcription
Oligo $(dT)_{12-18}$ primer is mainly used for synthesis of full-length cDNAs or preparation of whole cDNA libraries.

Hexamer primers* may be superior in RT-PCR in overcoming the difficulties encountered by secondary structure as well as in transcribing more 5' regions of the mRNA.

Sequence specific primers are widely used for cloning 5'-ends of mRNAs, as well as in RT-PCR when analyzing a specific target RNA or using a coupled RT-PCR reaction (one tube).

* available from Roche Diagnostics

Long Range RT-PCR with the Expand Long Template PCR System

For this application we recommend the use of Expand Long Template PCR System and the conditions indicated. Best results can be obtained by performing cDNA synthesis with a sequence specific reverse primer followed by using a different second reverse primer for the PCR reaction.

Especially important is:

- the use of $MgCl_2$ in a final concentration of 3 mM,
- the use of thin-walled 0.2 ml PCR tubes,
- setting up the reaction with two master mixes; always vortex when mix 1 and 2 are placed in the PCR tube together. Be careful to mix well, check that the entire volume is mixed but do not allow any mix to spill out. The vortexing should be done before mineral oil overlay,
- follow the cycle conditions as indicated.

General protocol for first-strand cDNA synthesis

Additional reagents required

Please refer to the ordering information for details.

- Oligo*, random or specific primers
- Water, PCR Grade*
- PCR Nucleotide Mix
- RNase Inhibitor, 40 U/µl (*e.g.* Protector RNase Inhibitor*)

Note: The use of RNase Inhibitor is not generally recommended. But the use of 20 U RNase Inhibitor for 20 µl reaction improves cDNA synthesis if contaminating RNases are present.

Procedure

Step	Action
1	Add reagents in the following order to a sterile RNase- and DNase- free preferably in 0.2 ml thin-walled PCR tube:

Component	Vol	Final conc.
Total RNA or Poly $(A)^+$ RNA	variable	1 µg total RNA or 50-100 ng Poly $(A)^+$ RNA
Primer	variable	Oligo$(dT)_{15}$ (20–100 pmoles) or random hexanucleotides (20–50 pmoles) or sequence specific reverse primer (10–50 pmoles)
Water, PCR Grade to a final volume of 10.5 µl	variable	

Step	Action
2	▪ Denature RNA and primer (from step 1) for 10 min at 65°C, preferably in a thermocycler with heated lid to avoid evaporation. ▪ Immediately cool on ice.
3	Add reagents in the following order, to the same PCR tube from step 1:

Component	Vol	Final conc.
5x Expand Reverse Transcriptase buffer (first-strand)	4 µl	1x
100 mM DTT	2 µl	10 mM
PCR Nucleotide Mix (10 mM each)	2 µl	1 mM each
RNase Inhibitor, 40 U/µl	0.5 µl	20 U
Expand Rev. Transcriptase 50 U/µl	1 µl	50 U
Total Volume	**20 µl**	

If you use...	Incubate PCR tube...
oligo $(dT)_{15-18}$ or sequ. spec. primers	45 - 60 min at 43°C
random hexamer primers	10 min at 30°C followed by 45 min at 42°C

4	Stop reaction by placing on ice.
5	Proceed to RT-PCR reactions or second-cDNA strand cDNA synthesis.
6	Purification of cDNA before PCR reaction is not necessary.

Heat inactivation of enzyme

Do not heat inactivate or freeze cDNA samples for applications in RT-PCR > 3 kb or second-strand cDNA synthesis.

For RT-PCR reaction up to 3 kb a heat inactivation of the enzyme by incubating at 95°C for 2 min is possible. In addition the cDNA reaction could be stored at +2 to +8°C or -15 to -25°C.

PCR amplification

Please follow the protocols given in the package insert of the appropriate enzyme. A selection guide of appropriate enzymes from Roche Applied Science for your application is given at the end of this package insert.

Results

Thermostable polymerases for PCR

When setting up a PCR, one should first determine which of the polymerases should be used. Main criteria for the detection is fidelity (6) and efficiency (*i.e.* yield and length) of the PCR.

Best yield in PCR can be obtained with Expand PCR Systems* followed by Taq DNA polymerase* and Tth DNA polymerase*.

Expand High Fidelity* can be used to amplify up to 10 kb fragments. Highest yield of fragments > 4 kb can be achieved by using the Expand Long Template PCR System*.

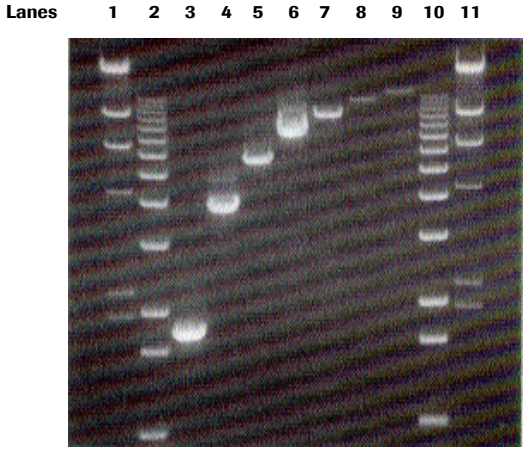

Fig 1: Amplification of cDNA fragments up to 13.6 kb from human dystrophin RNA using Expand Reverse Transcriptase and Expand Long Template PCR System.

One µg of total human muscle RNA was reverse transcribed with Expand Reverse Transcriptase. 5 µl of the unpurified cDNA was amplified with Expand Long Template PCR System.

17 µl of the PCR product was separated by gel electrophoresis.

Lane 1,11: DNA Molecular Weight Marker II*

Lane 2,10:Molecular Weight Marker X*

Lane 3: 1 857 bp; Lane 4: 4 041 bp; Lane 5: 5 893 bp; Lane 6: 7 678 bp

Lane 7: 9 556 bp; Lane 8: 11 962 bp; Lane 9: 13 482 bp

* available from Roche Diagnostics

Lanes 1 2 3 4 5 6 7 8 9 10 11,12,13 14,15,16 17

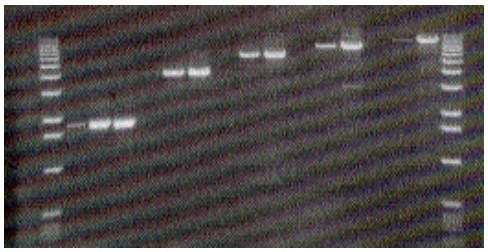

Fig 2: Amplification of dystrophin RNA by RT-PCR using Expand Reverse Transcriptase and different thermostable DNA polymerases.

One µg of total human muscle RNA was reverse transcribed with Expand Reverse Transcriptase. 5 µl of the unpurified cDNA was amplified with Pwo DNA polymerase, Expand High Fidelity PCR System or Expand Long Template PCR System, respectively. 17 µl of the PCR product was separated by gel electrophoresis.

Lane 1,17: DNA Molecular Weight Marker X*

Lane 2,3,4: 1 857 bp; Lane 5,6,7: 4 041 bp; Lane 8,9,10: 5 893 bp; Lane 11,12,13: 7 678 bp; Lane 14,15,16: 9 556 bp

Lane 2,5,8,11,14: Pwo DNA polymerase*

Lane 3,6,9,12,15: Expand High Fidelity PCR System*

Lane 4,7,10,13,16: Expand Long Template PCR System*

Quality control

For lot-specific certificates of analysis, see section, **Contact and Support**.

References

1 Houts, G.E. et al. (1979) *J. Virol.* **29**, 517-522.
2 Maniatis, T., Fritsch, E. F. & Sambrook, J. (1989) Molecular Cloning. A Laboratory Manual (Cold Spring Harbour Laboratory) p 8.11-8.13.
3 Gubler, U. & Hoffmann, B. J. (1983) *Gene* **25**, 263-269.
4 Sambrook, J. et al (1989) Molecular Cloning. A Laboratory Manual. Second Edition. (Cold Spring Harbour Laboratory) Part 7. and 8.
5 Rolfs, A. et al. (1992) PCR: Clinical Diagnostics and Research. Springer Verlag, page 99 - 111.
6 Cha, R.S. & Thilly, W.G. (1993) PCR Methods and Applications 3, p.18–29.
7 Ammendola, R. et al (2004) *Free Rad Bio Med* **36**,189-200.
8 Paradis, E. et al (2004) *Neurobiology of Disease* **15**, 312-325.

Ordering Information

PCR Product Selection Guide:

Needs	Size	Prevention of Carry over contamination*	Accuracy compared to Taq DNA Pol.	Use this RAS Science product	Pack size	Cat. No.
Standard PCR	up to 3 kb	Yes	1	Taq DNA Polymerase	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
			1	PCR Core Kit^{PLUS}	1 kit	11 585 541 001
		No	1	PCR Core Kit	1 kit	11 578 553 001
			1	PCR Master	1 kit	11 636 103 001
	up to 5 kb	Yes	6	Expand High Fidelity^{PLUS} PCR System	125 U 2× 250 U 10× 250 U	03 300 242 001 03 300 226 001 03 300 234 001
		No	3	Expand High Fidelity PCR System	100 U 2× 250 U 10× 250 U	11 732 641 001 11 732 650 001 11 759 078 001
			3	High Fidelity PCR Master	1 kit	12 140 314 001
Maximum specificity	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
High Fidelity PCR	up to 3 kb	No	18	Pwo SuperYield DNA Polymerase	100 U 2× 250 U	04 340 868 001 04 340 850 001
			18	Pwo Master	1 kit	03 789 403 001
			18	Pwo DNA Polymeras	100 U 2× 250 U	11 644 947 001 11 644 955 001
	up to 5 kb	No	3	Expand High Fidelity PCR System	100 U 2× 250 U 10× 250 U	11 732 641 001 11 732 650 001 11 759 078 001
			3	High Fidelity PCR Master	1 kit	12 140 314 001
		Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
			6	Expand High Fidelity^{PLUS} PCR System	125 U 2× 250 U 10× 250 U	03 300 242 001 03 300 226 001 03 300 234 001
Long Template PCR	5–20 kb	No	3	Expand Long Template PCR System	150 U 2× 360 U 10× 360 U	11 681 834 001 11 681 842 001 11 759 060 001
	> 20 kb	No	2	Expand 20 kb^{PLUS} PCR System	200 U	11 811 002 001
Difficult templates & challenging assays	up to 3 kb	Yes	1	FastStart Taq DNA Polymerase (Hot start)	50 U 100 U 500 U 4× 250 U 10× 250 U 20× 250 U	12 158 264 001 12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	up to 5 kb	Yes	4	FastStart High Fidelity PCR System (Hot start)	125 U 2× 250 U 10× 250 U	03 553 426 001 03 553 400 001 03 553 361 001
		No	3	GC-RICH PCR System	100 U	12 140 306 001
	5–20 kb	No	3	Expand Long Template PCR System	150 U 2× 360 U 10× 360 U	11 681 834 001 11 681 842 001 11 759 060 001

PCR Nucleotide Selection

Product	Characteristics	Pack size		Cat.No.
Set of Deoxy-Nucleotides, PCR Grade	Separate vials of dATP, dCTP, dGTP, and dTTP. 100 mM each	4× 25 µmol (4× 250 µl) 4× 125 µmol (4× 1250 µl)		11 969 064 001 03 622 614 001
Ready-to-use mixes of all 4 nucleotides				
PCR Nucleo-tide Mix PCR Grade, Na-Salt (10 mM each)	Premixed solution of dATP, dGTP, dCTP and dTTP, 10 mM each in water, pH 8.3		200 µl 2000 µl	11 581 295 001 11 814 362 001
PCR Nucleo-tide Mix ^{PLUS} PCR Grade, Na-Salt (10 mM each)	Premixed solution of dATP, dGTP, dCTP and dTTP, 10 mM each in water, pH 8.3		2× 200 µl	11 888 412 001

* available from Roche Applied Science

Additional Reagents

Product	Pack size	Cat. No.
Primer for cDNA Synthesis, $p(dT)_{15}$	1 A_{260} U	10 814 270 001
Primer for cDNA Synthesis, $p(dT)_{10}$	1 A_{260} U	10 814 261 001
Primer "random" $pd(N)_6$	50 A_{260} U	11 034 731 001
Protector RNase Inhibitor	2.000 U	03 335 399 001
DNase I recombinant RNase-free	10000 U	04 716 728 001
Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
Transcriptor Reverse Transcriptase	2000 U (4 × 500 U) for 200 reactions 500 U for 50 reactions 250 U for 25 reactions	03 531 287 001 03 531 295 001 03 531 317 001
Transcriptor First Strand cDNA Synthesis Kit	50 reactions	04 379 020 001
cDNA Synthesis System	1 kit (10 reactions)	11 117 831 001
5'/3' RACE Kit	1 kit (10 reactions)	03 353 621 001
High Pure RNA Isolation Kit	50 purifications	1 828 665 001
High Pure RNA Tissue Kit	50 purifications	12 033 674 001
TriPure Isolation Reagent	50 ml 250 ml	11 667 157 001 11 667 165 001
High Pure RNA Paraffin Kit	100 purifications	03 270 289 001
mRNA Isolation Kit	> 70 µg mRNA	11 741 985 001
mRNA Isolation Kit for Blood/Bone Marrow	30–100 purifications	11 934 333 001
High Pure Viral RNA Kit	100 purifications	11 858 882 001

Changes to Previous Version Update of the chapter Quality control.

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Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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