For life science research only.
Not for use in diagnostic procedures.

Expand Reverse Transcriptase
from *Escherichia coli* AP401 (k)

Version: 14
Content Version: December 2021

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase (RNA directed)

Cat. No. 11 785 826 001	1,000 U 20 µl (50 U/µl) *Not available in US*
Cat. No. 11 785 834 001	5,000 U 100 µl (50 U/µl) *Not available in US*

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Expand Reverse Transcriptase	Enzyme 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).	11 785 826 001	1 vial, 20 µl
			11 785 834 001	1 vial, 100 µl
2	Expand Reverse Transcriptase, Buffer for Expand Reverse Transcriptase, 5x conc.	First-strand cDNA synthesis buffer: 250 mM Tris-HCl, 200 mM KCl, 25 mM $MgCl_2$, 2.5% Tween 20 (v/v), pH 8.3 (+25°C).	11 785 826 001	1 vial, 1 ml
			11 785 834 001	1 vial, 1 ml
3	Expand Reverse Transcriptase, DTT 100 mM	Solution	11 785 826 001	1 vial, 1 ml
			11 785 834 001	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Expand Reverse Transcriptase	Store at −15 to −25°C.
2	Buffer for Expand Reverse Transcriptase, 5x conc.	
3	DTT 100 mM	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For first-strand cDNA synthesis

- Primers: oligo*, random*, or specific
- Template RNA
- Water, PCR Grade*
- PCR Nucleotide Mix*
- Protector RNase Inhibitor* (40 U/µl)

1.4. Application

Expand Reverse Transcriptase 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:

- Oligo $(dT)_{12-18}$ binds to endogenous poly(A)+ tail at the 3′ end of mammalian mRNA.
  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.
- Random hexanucleotides $(N)_6$ can bind to mRNA templates at any complementary site.
- Specific oligonucleotide sequences can be used to selectively prime the mRNA of interest.

The primary application of the Expand Reverse Transcriptase is the construction of libraries, 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 eukaryotic 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 eukaryotic genes.

2. How to Use this Product

2.1. Before you Begin

Primers

Priming reverse transcription

- Oligo $(dT)_{12-18}$ primer is primarily used for synthesis of full-length cDNAs and the preparation of whole cDNA libraries.
- Hexamer primers* may be superior for RT-PCR in overcoming the difficulties encountered by secondary structure as well as in transcribing the more 5′ mRNA regions.
- Sequence-specific primers are widely used for cloning 5′ ends of mRNAs, as well as in RT-PCR when analyzing a specific target RNA and using a coupled RT-PCR reaction (one tube).

General Considerations

RNA preparation

For high quality eukaryotic mRNA preparations, minimize the activity of RNases liberated during cell lysis using RNase inhibitors or methods that disrupt cells and inactivate RNases simultaneously. Suitable reagents for the isolation of total RNA or mRNA are the mRNA Isolation Kit* and TriPure Isolation Reagent*.

Avoid accidental introduction of RNases from other sources in the laboratory, such as glassware, plasticware, contaminating solutions, and especially, human hands.

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

RT-PCR

Upstream and downstream PCR primers are best selected from two exons spanning an intron. This allows the detection of DNA contamination of the RNA preparation because the PCR products will differ in size. If this is not possible, it will be necessary to treat the RNA preparation with DNase I recombinant*, RNase-free prior to RT-PCR.

Amount of cDNA

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

Integrity of mRNA

Integrity of mRNA is especially important when constructing cDNA libraries and when 5′ ends of mRNAs are investigated. mRNA size can be determined by gel electrophoresis and ethidium bromide staining. mRNA should appear as a smear, between approximately 500 bp and 8 kb in size. The bulk of the mRNA should be between 1.5 and 2 kb.

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

For this application, use the Expand Long Template PCR System*. Best results are obtained by performing cDNA synthesis using a sequence-specific reverse primer followed by a different second reverse primer for the PCR.
Of high importance is:

- Using $MgCl_2$ in a final concentration of 3 mM.
- Use of thin-walled 0.2 ml PCR tubes.
- Set up the reaction using two master mixes. Always vortex when mix 1 and 2 are placed in the PCR tube together. Mix well and check that the entire volume is mixed. Do not let any mix spill out of the tubes. Vortex before adding the mineral oil overlay.
- Follow the cycle conditions as indicated.

2.2. Protocols

First-strand cDNA synthesis

⚠ ***The use of RNase Inhibitor is not generally recommended, however 20 U RNase Inhibitor for a 20 µl reaction improves cDNA synthesis if contaminating RNases are present.***

1. To an autoclaved, RNase- and DNase-free 0.2 ml thin-walled PCR tube, add the components in the order listed for each 20 µl reaction:

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

2. Denature RNA and primer from Step 1 for 10 minutes at +65°C, preferably in a thermal cycler with heated lid to avoid evaporation.
   – Immediately cool on ice.

3. Add reagents in the order listed to the same PCR tube from Step 1:

Reagent	Volume [µl]	Final conc.
Buffer for Expand Reverse Transcriptase, 5x conc.	4	1x
DTT 100 mM	2	10 mM
PCR Nucleotide Mix* (10 mM each dNTP)	2	1 mM each
RNase Inhibitor*, 40 U/µl	0.5	20 U
Expand Reverse Transcriptase, 50 U/µl	1	50 U
Total Volume	**20**	

4. If you use oligo$(dT)_{15\text{-}18}$ or sequencing-specific primers, incubate PCR tube 45 to 60 minutes at +43°C.
   – Alternatively, if you use random hexamer primers, incubate PCR tube 10 minutes at +30°C followed by 45 minutes at +42°C.

5. Stop reaction by placing on ice.

6. Proceed to RT-PCR reactions or second-cDNA strand cDNA synthesis.

   ℹ *Purification of cDNA before PCR reaction is not necessary.*

PCR amplification

Follow the protocols in the Instruction for Use of the appropriate enzyme.

2.3. Parameters

EC-Number

EC 2.7.7.49

Inactivation

For RT-PCR reactions up to 3 kb, heat inactivate the enzyme by incubating at +95°C for 2 minutes. In addition, the cDNA reaction can be stored at +2 to +8°C or −15 to −25°C.

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

Unit Definition

One unit is the enzyme activity that incorporates 1.0 nmol TMP into acid insoluble products in 10 minutes at +37°C with poly(A)+ × $(dT)_{15}$ as substrate.

Volume Activity

50 U/µl

3. Results

Thermostable polymerases for PCR

When setting up a PCR, first determine which of the polymerases should be used. The main criteria for the detection is fidelity and efficiency, that is, yield and length of the PCR. Best yield in PCR can be obtained with Expand PCR Systems* (Figures 1 and 2) followed by Taq DNA Polymerase* and Tth DNA Polymerase*.
The Expand High Fidelity PCR System 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.

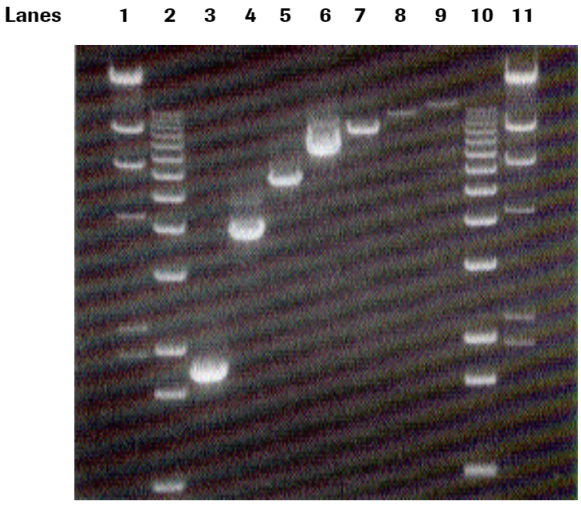

Fig. 1: Amplification of cDNA fragments up to 13.6 kb from human dystrophin RNA using Expand Reverse Transcriptase and Expand Long Template PCR System.
One µg of total human muscle RNA was reverse transcribed with Expand Reverse Transcriptase. 5 µl of the unpurified cDNA was amplified with the Expand Long Template PCR System. 17 µl of the PCR product was separated by gel electrophoresis.
Lanes 1 and 11: DNA Molecular Weight Marker II
Lanes 2 and 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

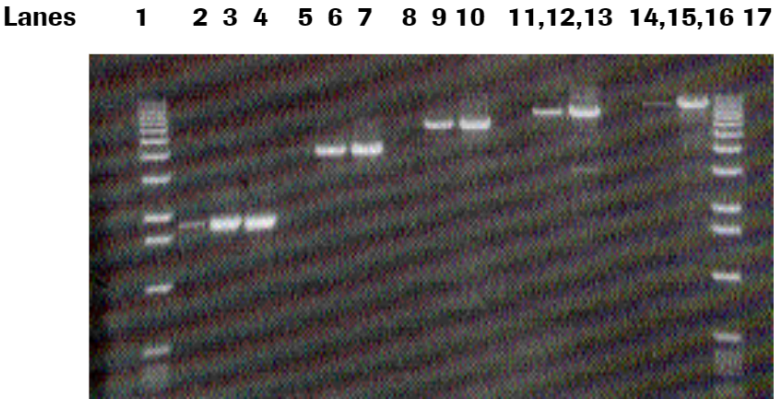

Fig. 2: Amplification of dystrophin RNA by RT-PCR using Expand Reverse Transcriptase and different thermostable DNA polymerases.
One µg of total human muscle RNA was reverse transcribed with Expand Reverse Transcriptase. 5 µl of the unpurified cDNA was amplified with Pwo DNA polymerase, Expand High Fidelity PCR System, or Expand Long Template PCR System, respectively. 17 µl of the PCR product was separated by gel electrophoresis.
Lanes 1 and 17: DNA Molecular Weight Marker X
Lanes 2, 3, and 4: 1,857 bp
Lanes 5, 6, and 7: 4,041 bp
Lanes 8, 9, and 10: 5,893 bp
Lanes 11, 12, and 13: 7,678 bp
Lanes 14, 15, and 16: 9,556 bp
Lanes 2, 5, 8, 11, and 14: Pwo DNA polymerase
Lanes 3, 6, 9, 12, and 15: Expand High Fidelity PCR System
Lanes 4, 7, 10, 13, and 16: Expand Long Template PCR System

4. Additional Information on this Product

4.1. Test Principle

Background information

Expand Reverse Transcriptase is a RNA-directed DNA polymerase optimized for amplification of cDNA fragments up to 13.5 kb in a two-step RT-PCR assay. The enzyme is a genetically engineered version of the Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV-RT). A point mutation within the RNase H sequence reduces RNase H activity to undetectable levels. Detectable improvements are obtained with respect to higher amounts of full-length cDNA transcripts and longer transcripts compared to using the native M-MuLV-RT.

4.2. Quality Control

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

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Primer for cDNA Synthesis	40 µg	10 814 270 001
Primer “random”	2 mg, 50 A_{260} units, 1 µmol 400 reactions à 5 µg primers	11 034 731 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
Expand Long Template PCR System	150 U, 1 x 150 U 38 reactions in a final volume of 50 µl	11 681 834 001
	720 U, 2 x 360 U 190 reactions in a final volume of 50 µl	11 681 842 001
	3,600 U, 10 x 360 U 950 reactions in a final volume of 50 µl	11 759 060 001
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001
Taq DNA Polymerase, 5 U/µl	100 U, 5 U/µl 80 reactions	11 146 165 001
	500 U, 5 U/µl 400 reactions	11 146 173 001
	4 x 250 U, 5 U/µl 800 reactions	11 418 432 001
	10 x 250 U, 5 U/µl 2,000 reactions	11 596 594 001
	20 x 250 U, 5 U/µl 4,000 reactions	11 435 094 001

Tth DNA Polymerase	500 U, 2 x 250 U, 5 U/µl	11 480 022 001
mRNA Isolation Kit	1 kit	11 741 985 001
TriPure Isolation Reagent	50 ml	11 667 157 001
	200 ml	11 667 165 001
Streptavidin Magnetic Particles	20 mg, 2 ml	11 641 778 001
	100 mg, 10 ml	11 641 786 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
Expand High Fidelity PCR System	100 U, 1 x 100 U 40 reactions in a final volume of 50 µl	11 732 641 001
	500 U, 2 x 250 U 200 reactions in a final volume of 50 µl	11 732 650 001
	2,500 U, 10 x 250 U 1,000 reactions in a final volume of 50 µl	11 759 078 001

5.4. Trademarks

EXPAND is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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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