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

1st Strand cDNA Synthesis Kit for RT-PCR (AMV)

For the first strand synthesis of single-stranded cDNA from RNA for use as a PCR template

Cat. No. 11 483 188 001
1 kit (30 reactions)

Version 10
Content version: October 2018
Store at −15 to −25°C

1. What This Product Does

Number of Tests
1 kit for 30 cDNA synthesis reactions (including 5 control reactions)

Kit Contents

Bottle	Label	Contents / Function
1	10× Reaction Buffer	1.050 ml 100 mM Tris, 500 mM KCl; pH 8.3
2	$MgCl_2$	3 vials, 1 ml each 25 mM
3	Deoxynucleotide Mix	210 µl dATP, dCTP, dTTP, dGTP; 10 mM each
4	Gelatin	210 µl 0.5 mg/ml (0.05% [w/v])
5	Oligo-p$(dT)_{15}$ Primer	60 µl 0.02 A_{260} units/µl (0.8 µg/µl)
6	Random Primer p$(dN)_6$	60 µl 0.04 A_{260} units/µl (1.6 µg/µl)
7	RNase Inhibitor	30 µl 50 units/µl
8	AMV Reverse Transcriptase	24 µl
9	Control Neo pa RNA	25 µl 0.2 µg/µl; 1.0 kb in length with an additional 19-base 3´-poly(A) tail
10	Water, PCR Grade	2 vials, 1 ml each

Storage and Stability
If properly stored, all kit components are stable until the expiration date printed on the label.
Avoid repeated freeze/thaw cycles for Control Neo pa RNA, RNase Inhibitor, and AMV Reverse Transcriptase.

Application
The First Strand cDNA Synthesis Kit is used for the synthesis of the first strand cDNA as the starting reaction for RT-PCR. It can be used with either sequence-specific primers, poly(dT)15 primers, or random primers, p(dN)6.
Thus, it can be used in combination with PCR for the detection of the presence or absence of RNA viruses or other RNA-containing microorganisms. Another application is the quantification of mRNA for monitoring differential expression of a specific mRNA. PCR products that are generated by RT-PCR can be cloned using standard procedures. This kit can also be used as the first step in the "differential display of mRNA".

2. How to Use This Product

2.1 Before You Begin

General Handling Recommendations
Please read all instructions before beginning the procedure.
To minimize the risk of RNase contamination, autoclave all vessels and pipette tips that will be used in the cDNA synthesis reaction. Gloves should be worn at all times.
Thaw Control Neo pa RNA, RNase Inhibitor, and AMV Reverse Transcriptase on ice. Thaw all other solutions at room temperature. Keep all reagents on ice after thawing. Briefly centrifuge all reagents before beginning the procedure.

2.2 Procedure

First Strand cDNA Synthesis

Because of the small volume of reagents required for each first strand cDNA synthesis reaction, we recommend that you prepare a larger amount of the Master Mix, centrifuge briefly, and subsequently aliquot into sample reaction tubes before adding the RNA sample. Preparation of this Master Mix will eliminate the need to repeatedly pipette small volumes, resulting in increased consistency between samples.

1 Mix the following components in a sterile microfuge tube:

Reagent	Volume / 1 sample	Final conc.	
10× Reaction Buffer	2.0 µl	1 ×	Master Mix
25 mM $MgCl_2$	4.0 µl	5 mM	
Deoxynucleotide Mix	2.0 µl	1 mM	
Primer (add **one** of the following)			
• Oligo-p$(dT)_{15}$ Primer, **or**	2.0 µl	0.04 A_{260} units (1.6 µg)	
• Random Primer p$(dN)_6$, **or**	2.0 µl	0.08 A_{260} units (3.2 µg)	
• Sequence-specific primer (not supplied)	variable	0.75 – 1 µM	
RNase Inhibitor	1.0 µl	50 units	
AMV Reverse Transcriptase	0.8 µl	≥20 units	
[a-^{32}P] dCTP, aqueous solution	optional [1]	10 – 20 µCi	
Gelatin	optional [2]	0.01 mg/ml	
Water, PCR Grade	variable	–	
RNA sample [3]	variable [4]	–	
Total	**20 µl**		

[1] To determine first strand cDNA yield, [α-^{32}P]dCTP may be added to the reaction mixture.
[2] Gelatin is an optional component of the first strand synthesis reaction (2).
[3] If extensive secondary structure is present in the RNA, the RNA sample may be denatured at +65°C for 15 min and placed on ice for 5 min before adding it to the reaction.
[4] The amount of RNA sample added to sample reactions depends on the nature of the RNA used and on the intended application; typically, ≤1 µg of total RNA, 50 – 100 ng of poly(A)$^+$ RNA, or 6 fg – 1 µg of a single, purified RNA species (*e.g.,* the Control Neo pa RNA) is required.

❷ Briefly vortex and centrifuge the mixture to collect the sample at the bottom of the microfuge tube.

❸ Incubate the reaction at +25°C for 10 min and then at +42°C for 60 min. During the first incubation, primer anneals to the RNA template. The RNA is subsequently reverse transcribed, resulting in cDNA synthesis during the second incubation.

❹ Following the +42°C incubation, the AMV Reverse Transcriptase may be denatured by incubating the reaction at +99°C for 5 min and then cooling to +4°C for 5 minutes.
If not denatured, AMV Reverse Transcriptase may interfere with subsequent applications (3).

At this point, the reaction tube may be stored at +2 to +8°C for 1 – 2 h or at −15 to −25°C for longer periods.

The quality and size of first strand cDNA products can be determined with gel electrophoresis on a denaturing alkaline agarose gel (4). Approximate size determinations can be made more easily with neutral agarose gels and NaOH sample denaturation (5).

Reverse Transcription PCR (RT-PCR)

The resulting single-stranded cDNA can be amplified in a polymerase chain reaction utilizing sequence-specific primers. This entire RT-PCR process (RNA to amplified cDNA) can be performed in a single tube without purifying the cDNA.

Following cDNA synthesis, prepare a cDNA amplification mixture by adding reagents to a sterile microfuge tube in the following order:

Because of the small volume of reagents required for each RT-PCR reaction, we recommend that you prepare a larger amount of the Master Mix, centrifuge briefly, and subsequently aliquot into reaction tubes containing cDNA.

❶ Mix the following components in a sterile microfuge tube:

Reagent	Volume/ 100 µl reaction	Volume/ Control Neo pa reaction	Final concentration
10× Reaction Buffer	8 µl	8 µl	1 ×
25 mM $MgCl_2$	variable	2 µl	1.5 mM [1]
Deoxynucleotide Mix	variable [2]	variable [2]	0.2 mM
Gelatin	optional 2 µl	optional 1 µl	0.01 mg/ml
Upstream Primer	1 µl	1 µl	0.2 µM [3]
Downstream Primer	1 µl [4]	1 µl [4]	0.2 µM [3]
Taq DNA Polymerase	0.5 µl	0.5 µl	2.5 units/100 µl
Water,PCR Grade	variable	65.5 µl	—
Template cDNA	variable [5]	20 µl [5]	<1 µg/100 µl
Total	**100.0 µl**	**100.0 µl**	

Alternatively, for maximum convenience in preparing the amplification reaction, try PCR Master [1], a PCR premix designed to minimize pipetting and potential sources of contamination. This 2× solution contains Taq DNA Polymerase (2.5 units/100 ml reaction), PCR nucleotide mix (0.4 mM of each nucleotide), and optimized reaction buffer (20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$, 0.2% [v/v] Brij 35). To use the PCR Master, mix the following in a sterile microcentrifuge tube:

Reagent	Volume/ 100 µl reaction	Final concentration
PCR Master	50 µl	2.5 U Taq DNA Polymerase; 0.005% (v/v) Brij 35; 10 mM Tris-HCl; 50 mM KCl; 1.5 mM $MgCl_2$[1]; dATP, dCTP, dGTP, dTTP, 0.2 mM each
Upstream Primer	1 µl	0.2 µM [3]
Downstream Primer	1 µl [4]	0.2 µM [3]
Water,PCR Grade	variable	—
Template DNA	variable [5]	<1 µg/100 µl
Total	**100 µl**	

Optimal reaction conditions for polymerase chain reactions vary significantly, depending on the template DNA and primers. To improve the yield and specificity of a specific amplification mixture, you may also want to modify the composition of the reaction buffer with the buffers and additives supplied in the PCR Optimization Kit [1] *.

This kit's sixteen buffers enable the simultaneous variation of pH range and $MgCl_2$ concentration in polymerase chain reactions. Further optimization can be achieved by testing the kit's other reaction additives. For example, dimethylsulfoxide can be used to reduce nonspecific priming (8 – 10). Gelatin and glycerol (11) stabilize Taq DNA polymerase, yielding more amplification product.

❷ Briefly vortex and centrifuge the mixture to collect the sample at the bottom of the microfuge tube.

❸ Cover the reaction mixture with 75 ml of mineral oil, and cycle. Amplification parameters depend greatly on the template and primers. For general information, see reference 6. Typically, cDNA is amplified for 35 or more cycles of denaturation, annealing, and polymerization with an additional extension time.

❹ Analyze 10-µl aliquots of the RT-PCR product by electrophoresis in an agarose gel, and stain with ethidium bromide (8). Expected results will appear as a single band (size determined by the primers).

[1] $MgCl_2$ concentrations may vary depending on the template, primer, and dNTP concentrations in the amplification reaction. To optimize conditions, use a $MgCl_2$ titration, generally between 0.5 and 10 mM (6).
[2] If the entire 20 µl product of the reverse transcription reaction is used, no additional dNTPs need to be added; however, if the product is divided for separate amplification experiments, dNTPs may have to be added to achieve a final concentration of 0.2 mM.
[3] Primer concentrations may vary; typical final concentrations range from 0.01 to 0.5 µM or 10 to 50 picomoles (6,7).
[4] If the sequence-specific downstream primer was used in reverse transcription, it does not need to be added to the PCR; only the corresponding upstream primer needs to be included in the PCR Master Mix.
[5] The amount of cDNA utilized in RT-PCR reactions may vary depending on the nature of the RNA template; typically, 5 µl of the cDNA of reverse transcribed total RNA, 20 µl of the cDNA resulting from reverse transcribed poly(A)$^+$ RNA, or 20 µl of the cDNA produced from a single, purified RNA species (*e.g.,* the Control Neo pa RNA) is used. If less than 20 µl of the cDNA reaction product is used, reagent volumes in the PCR Master Mix must be adjusted to achieve the dNTP and $MgCl_2$ final concentrations specified above.

3. Results

3.1 RT-PCR Function Testing

In the standard RT-PCR assay, varying amounts (10^{12}, 10^6, 10^5, or 10^4 molecules; corresponding to 1 µg, 600 fg, 60 fg, or 6 fg, respectively) of Control Neo pa RNA template are reversely transcribed, then amplified utilizing primers specific for a 382 bp fragment
(upstream primer: 5′ GCT CTG ATG CCG CCG TGT 3′;
downstream primer: 5′ CCC TGA TGC TCT TCG TCC 3′).
Amplification parameters for the Control Neo pa RNA:

Denaturation	96°C, 1 minute
Annealing	57°C, 1 minute
Polymerization (35 cycles)	72°C, 2 minutes
Link; Extension (1 cycle)	70°C, 5 minutes

Link to a 4°C Soak file.

Upon gel electrophoresis, the 382 bp fragment determined by the primers is observed (Figure 1).

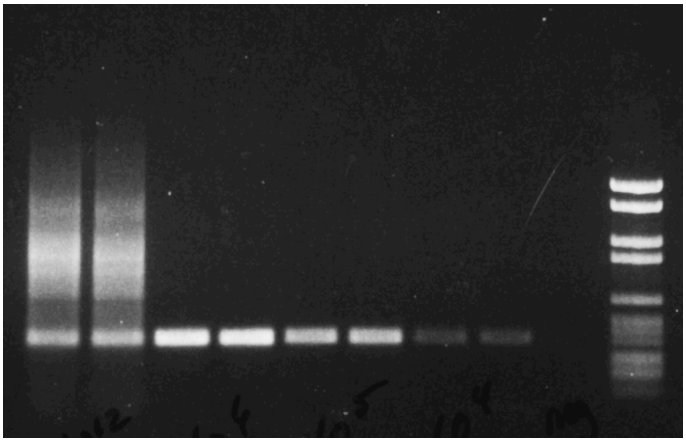

Fig. 1: RT-PCR of the Control Neo pa RNA. Varying amounts (10^{12} [Lanes 1 and 2], 10^6 [Lanes 3 and 4], 10^5 [Lanes 5 and 6], or 10^4 [Lanes 7 and 8] starting molecules) of the Control Neo pa RNA template are reverse transcribed and then amplified with primers specific for a 382 bp fragment (upstream primer: 5′ GCT CTG ATG CCG CCG TGT 3′; downstream primer: 5′ CCC TGA TGC TCT TCG TCC 3′). Note that the amount of RNA in Lanes 1 and 2 is so great that high molecular weight smears are evident. Lane 9 contains a negative control. Lane 10 contains DNA Molecular Weight Marker VI.

3.2 First Strand cDNA Synthesis Function Testing

Typically, in the standard cDNA synthesis assay, at least 300 ng of cDNA is synthesized (*i.e.,* ≥30% yield) when 1.0 µg Control Neo pa RNA template is incubated with 20 µCi [a-^{32}P] dCTP (specific activity of 3,000 Ci/mmol) for 60 min at +42°C.

3.3 Calculation

Use the following formulas to calculate first strand cDNA yield and % yield:

$$\text{cDNA yield (µg)} = \left(\frac{\text{cpm incorporation}}{\text{cpm input}}\right) \times \left(\text{mol dCTP in the assay}\right) \times [4 \text{ (no. dNTP)}] \times [330 \text{ g/mol (dNTP MW)}] \times \left(\frac{10^6 \text{ µg}}{1 \text{ g}}\right)$$

$$\% \text{ yield} = \frac{\text{µg cDNA}}{\text{µg Template}} \times 100$$

For example, in a 20 µl assay with 20 µCi [α-^{32}P] dCTP:

$$\left[\frac{9.51 \times 10^2 \text{ cpm}}{6.41 \times 10^4 \text{ cpm}}\right] \times (2 \times 10 - 8 \text{ mol dCTP})^{1} \times (4) \times (330 \text{ g/mol}) \left(\frac{(10^6 \text{ µg})}{1 \text{ g}}\right) = 0.392 \text{ µg cDNA yield}$$

1 This represents the amount of unlabeled dCTP nucleotide present in the reaction, as the amount of labeled dCTP added to the reaction is insignificant.

4. Additional Information on this Product

Background Information

Using the 1st Strand cDNA Synthesis Kit, RNA is reverse transcribed into single-stranded cDNA (1). In this method, AMV Reverse Transcriptase synthesizes the new cDNA strand at a site(s) determined by the type of primer used: at the 3′-end of the poly(A)-mRNA when Oligo-p$(dT)_{15}$ is used as a primer, at nonspecific points along the RNA template when using the Random Primer p$(dN)_6$, or at a primer-binding site for a sequence-specific primer. The resulting first strand cDNA can then be used as a template for PCR.

AMV Reverse Transcriptase is well suited to the preparation of cDNA for use as a PCR template. Fully active in PCR buffer, AMV Reverse Transcriptase features a high specific activity, ensuring large amounts of template. At 42°C, this highly processive enzyme is more efficient than M-MuLV Reverse Transcriptase.

Quality Control

In the standard RT-PCR assay, varying amounts of Control Neo pa RNA template are reversely transcribed, then amplified utilizing primers specific for a 382 bp fragment. Each lot is tested for RNase and DNase activity.

References

1 Sambrook, J., Fritsch, E.M. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 8.11–8.13.

2 Shuldiner, A.R., Tanner, U., Moore, C.A. and Roth, J. (1991) *BioTechniques* **11**, 760–763.

3 Kawasaki, E.S. (1990) "*Amplification of RNA" in PCR Protocols: A Guide to Methods and Applications* (Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., eds.) p. 23, Academic Press, San Diego, CA.

4 Sambrook, J., Fritsch, E.M. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 6.20, 8.5.

5 Lenstra, J.A., DeGroot, R.J., Jacobs, L., Kustens, J.G., Niesters, H.G.M. and Van Der Zeijst, B.A.M. (1988) *Gene Anal. Techn.* **5**, 57–61.

6 Erlich, H.A., ed. (1989) *PCR Technology: Principles and Applications for DNA Amplification,* Stockton Press, New York.

7 Sambrook, J., Fritsch, E.M. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 14.16, 14.20.

8 Pomp, D. and Medrano, J.F. (1991) *BioTechniques* **10**(1), 58–59.

9 Filichkin, S.A. and Gelvin, S.B. (1992) *BioTechniques* **12**(6), 828–830.

10 Masoud, S.A., Johnson, L.B. and White, F.F. (1992) *PCR Methods and Applications* **2**, 89–90.

11 Rolfs, A. *et al.* (1992) PCR: *Clinical Diagnostics and Research*, Springer Laboratory, pp. 51–67.

12 Sambrook, J., Fritsch, E.M. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 6.3–6.23.

5. Supplementary Information

5.1 Conventions

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Symbol	Description
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered Instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

5.2 Changes to Previous Version

Editorial changes.

5.3 Trademarks

All third party product names and trademarks are the property of their respective owners.

5.4 Regulatory Disclaimer

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

5.5 Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

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.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany