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

First Strand cDNA Synthesis Kit for RT-PCR (AMV)

Version: 11
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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, including 5 control reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Reaction Buffer, 10x conc.	Buffer composition: 100 mM Tris-HCl, 500 mM KCl, pH 8.3.	1 vial, 1.05 ml
2	First Strand cDNA Synthesis Kit for RT-PCR (AMV), $MgCl_2$ Stock solution, 25 mM	For optimization of Mg^{2+} concentration.	3 vials, 1 ml each
3	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Deoxynucleotide Mix	dNTP stock solution, containing 10 mM dATP, dCPT, dGTP, dTTP each.	1 vial, 210 µl
4	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Gelatin	0.5 mg/ml (0.05% [w/v])	1 vial, 210 µl
5	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Oligo $p(dT)_{15}$ Primer	0.02 A_{260} U/µl (0.8 µg/µl)	1 vial 60 µl
6	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Random Primer $p(dN)_6$	0.04 A_{260} U/µl (1.6 µg/µl)	1 vial 60 µl
7	First Strand cDNA Synthesis Kit for RT-PCR (AMV), RNase Inhibitor	50 U/µl	1 vial 30 µl
8	First Strand cDNA Synthesis Kit for RT-PCR (AMV), AMV Reverse Transcriptase	Synthesis of first-strand cDNA for use in subsequent amplification reactions.	1 vial 24 µl
9	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Control Neo pa RNA	0.2 µg/µl; 1.0 kb in length with additional 19-base 3′-poly(A) tail.	1 vial 25 µl
10	First Strand cDNA Synthesis Kit for RT-PCR (AMV), Water, PCR Grade	To adjust final reaction volume.	2 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	Reaction Buffer, 10x conc.	Store at −15 to −25°C.
2	$MgCl_2$ Stock solution, 25 mM	
3	Deoxynucleotide Mix	
4	Gelatin	
5	Oligo $p(dT)_{15}$ Primer	
6	Random Primer $p(dN)_6$	
7	RNase Inhibitor	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
8	AMV Reverse Transcriptase	
9	Control Neo pa RNA	
10	Water, PCR Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile 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

- Template RNA
- α^{32}P-dCTP, aqueous solution
- Sequence-specific primer (optional)

For RT-PCR

- PCR Master* (optional)
- Template DNA
- Mineral oil (optional)

1.4. Application

The First Strand cDNA Synthesis Kit for RT-PCR (AMV) is used for the synthesis of the first strand cDNA as the starting reaction for RT-PCR.

- Use with either sequence-specific primers, poly(dT)$_{15}$ primers or random primers, p(dN)$_6$.
- Combine with PCR for the detection of the presence or absence of RNA viruses or other RNA-containing microorganisms.
- For 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.
- Use as the first step in the differential display of mRNA.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

First strand cDNA synthesis

For template RNA, use:

- ≤1 µg of high quality intact total RNA
- 50 to 100 ng of isolated, purified poly(A)$^+$ mRNA
- 6 fg to 1 µg of a single, purified RNA species, such as the Control Neo pa RNA

Reverse transcription PCR (RT-PCR)

The resulting single-stranded cDNA can be amplified in a PCR using sequence-specific primers.

Primers

Reverse transcription PCR (RT-PCR)

Primer concentrations may vary; typical final concentrations range from 0.01 to 0.5 µM or 10 to 50 picomoles. If a sequence-specific reverse primer was used in reverse transcription, this primer does not need to be added again to the PCR; only the corresponding forward primer needs to be included in the PCR Master Mix.

Mg2+ Concentration

Reverse transcription PCR (RT-PCR)

$MgCl_2$ concentrations may vary depending on the template, primer, and dNTP concentrations in the amplification reaction. To optimize conditions, perform a $MgCl_2$ titration, generally between 0.5 and 10 mM.

General Considerations

Precautions

To minimize the risk of RNase contamination:
- Autoclave all vessels and pipette tips that will be used in the cDNA synthesis reaction.
- Wear gloves at all times.
- Thaw Control Neo pa RNA, RNase Inhibitor, and AMV Reverse Transcriptase on ice.
- Thaw all other solutions at +15 to +25°C; keep all reagents on ice after thawing.
- Briefly centrifuge all reagents before beginning the procedure.

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Working Solution

First strand cDNA synthesis

Due to the small volume of reagents required for each first strand cDNA synthesis reaction, 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.

Reverse transcription PCR (RT-PCR)

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

2.2. Protocols

First strand cDNA Synthesis

See section, **Working Solution** *for information on preparing master mixes.*

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. To a sterile 1.5 ml tube on ice, add the components in the order listed:
   The Master Mix consists of the first 6 Reagents, containing only the Reaction Buffer, $MgCl_2$, Deoxynucleotide Mix, Primers, RNase Inhibitor, and AMV Reverse Transcriptase.

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

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

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

5. Denature the AMV Reverse Transcriptase by incubating the reaction at +99°C for 5 minutes and then cooling to +4°C for 5 minutes.
   – If not denatured, the AMV Reverse Transcriptase may interfere with subsequent applications.

6. Store reaction tube at +2 to +8°C for 1 to 2 hours or at −15 to −25°C for longer periods.

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

(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.
(3) If extensive secondary structure is present in the RNA, the RNA sample may be denatured at +65°C for 15 minutes and placed on ice for 5 minutes 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 to 100 ng of poly(A)$^+$ RNA, or 6 fg to 1 µg of a single, purified RNA species, such as the Control Neo pa RNA is required.

Reverse transcription PCR (RT-PCR) protocol 1

See section, **Working Solution** *for information on preparing master mixes.*

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

1. Following cDNA synthesis, prepare a cDNA amplification mixture by adding the following components to a sterile microfuge tube in the order listed:

   ⚠ ***Alternatively, for maximum convenience in preparing the amplification reaction, use the PCR Master*, see section, Reverse transcription PCR (RT-PCR) protocol 2***

Reagent	Volume 100 µl reaction [µl]	Volume Control neo pa reaction [µl]	Final conc.
Reaction Buffer, 10x conc.	8	8	1x
$MgCl_2$, 25 mM	variable	2	1.5 mM(1)
Deoxynucleotide Mix	variable(2)	variable(2)	0.2 mM
Gelatin	2 (optional)	1 (optional)	0.01 mg/ml
Forward primer 1	1	1	0.2 µM(3)
Reverse primer 2	1(4)	1(4)	0.2 µM(3)
Taq DNA Polymerase	0.5	0.5	2.5 U/reaction
Water, PCR Grade	add up to a final volume of 100	65.5	–
Template cDNA	variable(5)	20(5)	<1 µg/100 µl
Final Volume	**100**	**100**	

   Optimal reaction conditions for PCR vary significantly, depending on the template DNA and primers. To improve the yield and specificity of a specific amplification mixture, you can modify the composition of the reaction buffer with additives. For example, dimethylsulfoxide can be used to reduce nonspecific priming. Gelatin and glycerol stabilize Taq DNA Polymerase, yielding more amplification product.

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

3. If the thermal cycler does not have a top heater, overlay the reaction with 75 µl mineral oil.

4. Place samples in a thermal block cycler, and perform PCR.
   – Amplification parameters depend greatly on the template and primers.
   Typically cDNA is amplified for ≥35 cycles of denaturation, annealing, and polymerization with an additional extension time.

5. Analyze 10 µl aliquots of the RT-PCR product by agarose gel electrophoresis followed by ethidium bromide staining.
   – 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.

(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 must be added to achieve a final concentration of 0.2 mM.

(3) Primer concentrations may vary; typical concentrations range from 0.01 to 0.5 µM or 10 to 50 picomoles.

(4) If the sequence-specific reverse primer was used in reverse transcription, it does not need to be added to the PCR; only the corresponding forward 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, such as the Control Neo pa RNA is used. If <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 in the protocol.

Reverse transcription PCR (RT-PCR) protocol 2

See section, **Working Solution** *for information on preparing master mixes.*

For maximum convenience in preparing the amplification reaction, use the PCR Master*[1]. This PCR premix minimizes pipetting and potential sources of contamination. The 2x solution contains Taq DNA Polymerase (2.5 U/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).

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

1. Following cDNA synthesis, prepare a cDNA amplification mixture by adding the following components to a sterile microfuge tube in the order listed:

Reagent	Volume 100 µl reaction [µl]	Final conc.
PCR Master	50	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], 0.2 mM each dATP, dCTP, dGTP, dTTP.
Forward primer 1	1	0.2 µM[2]
Reverse primer 2	1[3]	0.2 µM[2]
Water, PCR Grade	add up to a final volume of 100	–
Template DNA	variable[4]	<1 µg/100 µl
Final Volume	**100**	

Optimal reaction conditions for PCR vary significantly, depending on the template DNA and primers. To improve the yield and specificity of a specific amplification mixture, you can modify the composition of the reaction buffer with additives. For example, dimethylsulfoxide can be used to reduce nonspecific priming. Gelatin and glycerol stabilize Taq DNA Polymerase, yielding more amplification product.

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

3. If the thermal cycler does not have a top heater, overlay the reaction with 75 µl mineral oil.

4. Place samples in a thermal block cycler, and perform PCR.
   – Amplification parameters depend greatly on the template and primers.
   Typically cDNA is amplified for ≥35 cycles of denaturation, annealing, and polymerization with an additional extension time.

5. Analyze 10 µl aliquots of the RT-PCR product by agarose gel electrophoresis followed by ethidium bromide staining.
   – 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.

(2) Primer concentrations may vary; typical concentrations range from 0.01 to 0.5 µM or 10 to 50 picomoles.

(3) If the sequence-specific reverse primer was used in reverse transcription, it does not need to be added to the PCR; only the corresponding forward primer needs to be included in the PCR Master Mix.

(4) 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, such as the Control Neo pa RNA is used. If <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 in the protocol.

3. Results

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 reverse transcribed, then amplified using primers specific for a 382 bp fragment.
Forward primer: 5′ GCT CTG ATG CCG CCG TGT 3′
Reverse 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).

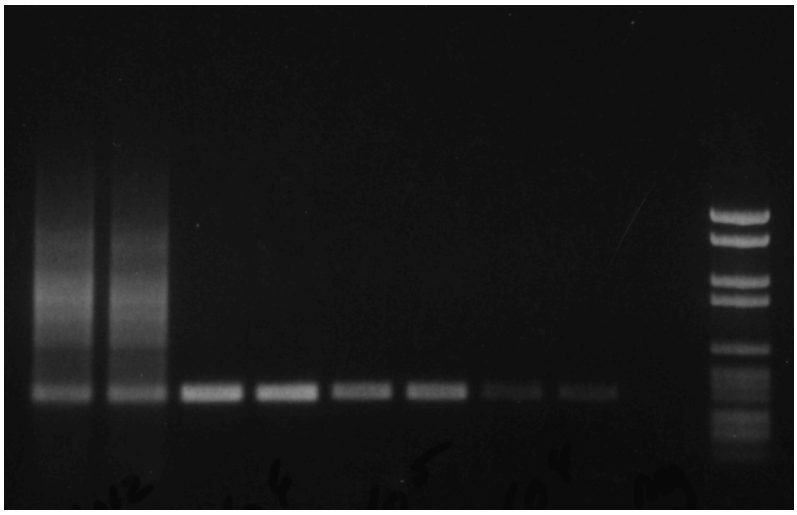

Fig. 1: RT-PCR of the Control Neo pa RNA. Varying amounts (10^{12} **[Lanes 1 and 2]**, 10^{6} **[Lanes 3 and 4]**, 10^{5} **[Lanes 5 and 6]**, or 10^{4} **[Lanes 7 and 8]** starting molecules) of the Control Neo pa RNA template are reverse transcribed and then amplified with primers specific for a 382 bp fragment (forward primer: 5′ GCT CTG ATG CCG CCG TGT 3′; reverse 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: Negative control
Lane 10: DNA Molecular Weight Marker VI

First strand cDNA synthesis function testing

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

Calculation

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

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

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

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

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

(1) 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

4.1. Test Principle

Using the First Strand cDNA Synthesis Kit for RT-PCR, RNA is reverse transcribed into single-stranded cDNA. 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)$_6$,
- 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.

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.
New information added related to the REACH Annex XIV.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Master	1 kit, 100 reactions in a final volume of 100 µl	11 636 103 001
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

5.4. Trademarks

All 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.

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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