Steinbrenner Laborsysteme GmbH

primaREVERSE RT-KIT

Reverse Transcription Kit

Article	Content
SL-9540-smp	20 rxn × 20 µl
SL-9540-100	100 rxn × 20 µl
SL-9540-1000	1000 rxn × 20 µl

Long-Term Storage
at -20°C in the dark

Short-Term Storage
at 4°C in the dark

DESCRIPTION

Our **primaREVERSE RT-KIT** contains all necessary components for a reverse transcription of RNA/mRNA to cDNA. It also includes a proprietary MuLV enzyme for fast and specific generation of cDNA and an enzyme mix for the efficient removal of genomic DNA.

Content	9 tubes (including DNase mix, water, primers)
Reverse Transcriptase	proprietary, reduced RNaseH activity
RNA Input	up to 10 ug per reaction
Amplicon length	up to 17 kb
RT time	5 – 30 minutes
gDNA removal	included, optional
Primer	Oligo(dT)$_{20}$ (included), Random Hexamers (included), Gene-Specific Primers

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Workflow

Standard Workflow

1. Prepare 20 µl **Reaction Mix** with RNA template
2. **Reverse Transcription**
   10 minutes at 50°C
3. **Inactivation**
   2 minutes at 95°C

Extended Workflow

OPTIONAL: Perform gDNA Removal

1. Prepare 10 µl **Reaction Mix** with RNA Template
2. **gDNA Removal**
   5 – 10 minutes at 37°C
3. **Inactivation**
   2 minutes at 95°C

1. Prepare 10 µl **Primer Mix** with RNA template
2. Incubate for 5 minutes at 70°C
3. Prepare 10 µl **Final Reaction Mix** and add it to the **Primer Mix**
4. **Reverse Transcription**
   10 minutes at 50°C
5. **Inactivation**
   2 minutes at 95°C

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Included Reagents

Cap Colour	Component	Stock Concentration
Green	DNase Enzyme Mix	5 U/µl
Yellow	10x DNase Incubation Buffer	10x
Orange	Reverse Transcriptase	200 U/µl
Brown	5x RT Reaction Buffer	5x
Transparent	dNTP Mix	10 mM
White	Oligo(dT)$_{20}$ Primer	100 µM (100 pmol/µl)
Pink	Random Hexamer Primer	100 µM (100 pmol/µl)
Purple	RNase Inhibitor	40 U/µl
Blue	RNase-free Water	–

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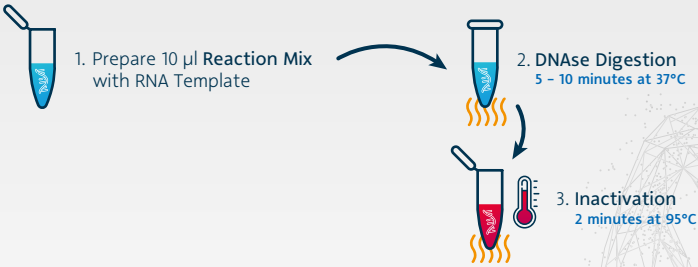
Optional: gDNA Removal Protocol

Digestion of residual genomic DNA **improves the quality** of cDNA synthesis and subsequent applications. The removal of gDNA is recommended for samples if **only RNA** is to be analyzed after reverse transcription.

This is especially true for PCR/qPCR assays that are performed with primers/ probes that are not intron- / or exon-spanning.

DNAse Digestion



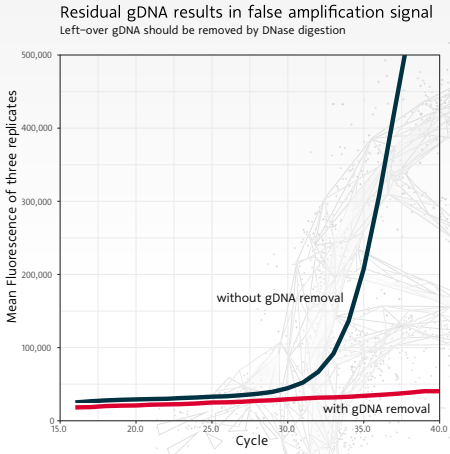


Figure 1:
Residual gDNA can be amplified during RT-qPCR

PREPARE DNASE I REACTION MIXTURE

NOTE

Keep all reagents on ice.

Component	Stock Concentration	10 µl Final Reaction Mix	Final Concentration
10x DNase Incubation Buffer	10x	1 µl	1x
DNase Enzyme Mix	5 U/µl	1 µl	0.5 U/µl
RNA Sample	variable	0.1 - 2 µg	variable
RNase-free Water	–	adjust to 10µl	–

DIGESTION PROTOCOL

Step	Time	Temperature
Digestion	5 - 10 minutes	37°C
Inactivation	2 minutes	95°C

NOTE

Spin down and use directly for cDNA synthesis or place on ice

REVERSE TRANSCRIPTION

For the Standard Protocol please continue with page 6, for the Extended Protocol please go to page 8.

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Recommended: Standard Protocol

NOTE

Keep all reagents on ice.

Reverse Transcription

PREPARE REACTION MIXTURE

- Add all reagents according to page 7 and fill up with **RNase-free water** to a total volume of 20 µl.
- Proceed with the reverse transcription reaction as shown on **page 10**.

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Recommended: Standard Protocol

Component	Stock Concentration	20 µl reaction	Final Concentration
RNA Template or DNase-digested Sample*	–	10 µl	10 pg to 5 µg/reaction*
Primer	100 µM	1 µl	5 µM** Gene-Specific Primer: 500 nM***
5x RT Reaction Buffer	5x	4 µl	1x
dNTP Mix	10 mM	1 µl	500 µM
RNase Inhibitor (optional)	40 U/µl	0.5 µl	1 U/µl
Reverse Transcriptase	200 U/µl	1 µl	10 U/µl
RNase-free Water	–	adjust to 20 µl	–

* Total RNA: 10 pg - 5 µg; purified mRNA: 10 pg - 500 ng; Sample from DNase Digestion Step

** either Oligo(dT)$_{20}$, Random Hexamer or 1:1 mix of Oligo(dT)$_{20}$ and Random Hexamer Primer

*** For Gene-Specific Primers we recommend to use 500 nM final primer concentration

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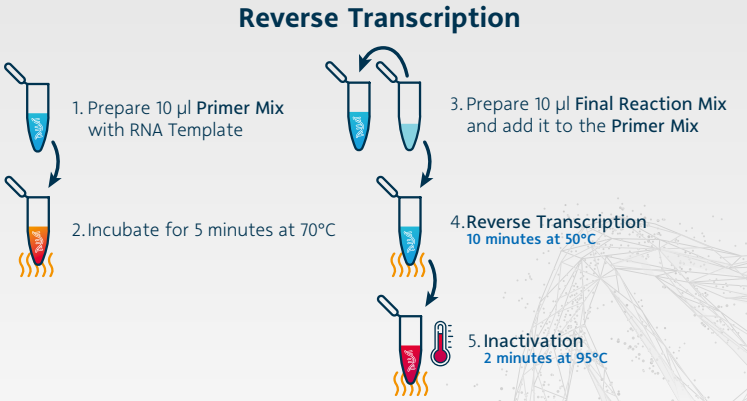
Optional: Extended Protocol

NOTE

Keep all reagents on ice. The extended protocol is recommended for RNA targets with a high degree of secondary structure.



1. PREPARE PRIMER/RNA MIX

Prepare the primer/RNA mix and gently mix the reaction.

Component	Stock Concentration	11 µl reaction	Final Concentration
RNA Template or DNase digested Sample*	–	variable (up to 10 µl)	10 pg to 5 µg/reaction*
Primer**	100 µM	1 µl	5 µM**, Gene-Specific Primer: 500 nM***
RNase-free Water	–	adjust to 11 µl	–

* Total RNA: 10 pg - 5 µg; purified mRNA: 10 pg - 500 ng; Sample from DNase Digestion Step

** either Oligo(dT)$_{20}$, Random Hexamer or 1:1 mix of Oligo(dT)20 and Random Hexamer Primer

*** For Gene-Specific Primers we recommend to use 500 nM final primer concentration

2. PRIMER ANNEALING

- After incubation, place on ice.
- Proceed with the preparation of the final reaction mix below.

Step	Time	Temperature
Incubation	5 minutes	70°C

3. PREPARE FINAL REACTION MIX

- Prepare the reaction mix as shown below.
- Add 10 µl of the prepared reaction mix to the Primer/RNA mix of Step 2.
- Gently mix the tube and proceed with the reverse transcription on **page 10**.

Component	Stock Concentration	9 µl Reaction Mix	Final Concentration in 20 µl
5x Reaction Buffer	5x	4 µl	1x
dNTP Mix	10 mM	1 µl	500 µM
RNAse Inhibitor (optional)	40 U/µl	0.5 µl	1 U/µl
Reverse Transcriptase	200 U/µl	1 µl	10 U/µl
RNAse-free Water	–	adjust to 9 µl	–

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Reverse Transcription

NOTE

The time for the reverse transcription reaction depends on the cDNA length. Increase the temperature to 55°C for difficult templates.

Step	Time	Temperature
Reverse Transcription	10 minutes	50°C
Optional: Heat Inactivation	2 minutes	95°C

- Reverse-transcripted cDNA should be stored at -20°C.
- Avoid frequent freeze/thaw of the reagents and the final cDNA.
- Do not use more than 5 µl for downstream applications such as qPCR.
- Difficult and long templates can be run for 30-60 min to increase yield and cDNA length.

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Additional Information

APPLICATIONS

- Synthesis of cDNA from RNA templates

QUALITY CONTROL PROCEDURE

Our **primaREVERSE RT-KIT** undergoes stringent quality controls. Each lot is verified with RNA input; enzyme purity and homogeneity is > 98 %.

All reagents are verified free of detectable endonuclease- & exonuclease activity.

FURTHER INFORMATION

For more information, please visit our website: **www.steinbrenner.de**

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Further Products

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