Reverse Transcriptase 200 U/µl, with glycerol

Description: Reverse Transcriptase is a M–MuLV enzyme for fast and specific generation of cDNA. This polymerase can synthesize a complementary DNA strand using either RNA or single-stranded DNA as template. Reverse Transcriptase is genetically modified for reduced RNase H activity, which improves cDNA synthesis. The enzyme shows optimal activity at 50 – 55 °C. Reverse Transcriptase is supplied with optimized Reaction Buffer for best results.

Concentration: 200 units/µl

Storage: -18 °C to – 22 °C

REF	105550	105500	colour
Reverse Transcriptase, with glycerol	**10 000 units**	**100 000 units**	**blue**
5x Complete Reaction Buffer	**2x 2 ml**	**40 ml**	**yellow**
MgCl2, 100 mM	**1 ml**	**5x 1 ml**	**green**

Application: First-strand synthesis of cDNA from RNA or single-stranded DNA. Reverse Transcriptase shows a synthesis capacity up to 13 kb cDNA product, which can be amplified by PCR with Taq Polymerases afterwards. The enzyme is highly suitable for the use in One Step RT-qPCR reactions.

Unit definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)/ oligo(dT) as template primer.

Quality Control:

- enzyme purity corresponds to ≥ 98% homogeneity by SDS-PAGE
- no detectable exo-/endonuclease and RNase activities

Please see page 2 for recommended standard protocol for Reverse Transcription.

- Avoid frequent freeze/thaw of the reagents and 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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Recommended Standard Protocol:

Component	20 µl Reaction	Final Concentration
Sample	10 µl	total RNA: 10 pg - 5 µg purified mRNA: 10 pg – 500 ng
Primer (100 µM)	1 µl	5 µM *
5x Reaction Buffer	4 µl	1x
dNTP Mix (10 mM)	0.5 µl	500 µM
Optional: RNase Inhibitor (40 U/µl)	1 µl	1 U/µl
Reverse Transcriptase 200 U/µl	1 µl	10 U/µl
RNase-free Water	adjust to 20 µl final volume	--

* Oligo(dT) Primer or Random Primer or a 1:1 mix of both primers. For gene specific primers we recommend 500 nM final primer concentration.

Thermocycler protocol

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

* The time for reverse transcription reaction depends on the cDNA length. For difficult templates please increase the temperature to 55 °C.

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