

Data Sheet (02.11.2007, NV)

mi-Reverse Transcriptase (H-)

(M-MuLV, RNase H minus)

Cat.-No.	Size	Conc.
mi-E8100	10000 units	200 u/μl

Description

mi-Reverse Transcriptase (H-) (Moloney Murine Leukemia Virus Reverse Transcriptase RNase H minus) is an RNA-directed DNA polymerase. The enzyme synthesizes a complementary DNA strand initiating from a primer using single-stranded RNA or DNA as template. M-MuLV RT RNase H⁻ is genetically modified to eliminate its RNase H activity. Removal of the RNase H activity results in an increase of full-length cDNA products.

Unit definition

One unit of activity is the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 min at 37 °C, using polyA-oligo as a template.

M-MuLV Reverse Transcriptase (red cap)

50 mM TrisHCl (pH 8.0), 100 mM NaCl, 5 mM DTT, 1 mM EDTA, 0.1% NP-40, 50 % glycerol (v/v)

Supplied 5x RT buffer "complete" (green cap)

250 mM TrisHCl (pH 8.3), 500 mM KCl, 30 mM MgCl₂, 25 mM DTT

Supplied 5x RT buffer "incomplete" (blue cap)

250 mM TrisHCl (pH 8.3), 500 mM KCl

MgCl₂ stock solution (yellow cap)

25 mM MgCl₂

MnCl₂ stock solution (white cap)

20 mM MnCl₂

DTT stock solution (purple cap)

100 mM DTT

Storage at -20 °C, for *in vitro* use only

Recommended cDNA synthesis protocol

- mix 1-5 μg RNA with
 - 250-500 ng oligo-dT₁₅₋₂₅ or 50-100 ng random primers / gene-specific primer per μg of RNA,
 - 1-2 μl dNTP Mix (10 mM of each dNTP),
 - add nuclease-free water to 20 μl.
- Incubate the mixture at 70 °C for 5-10 min and place it at room temperature for 10-15 min (if using a specific primer) or on ice (if using oligo-dT or random primer). Centrifuge the tube and add:
 - 10 μl 5x Reaction Buffer complete (for individual optimization of the assay conditions use 5x Reaction Buffer without MgCl₂/DTT and add the required amounts of MgCl₂ (6-12 μl) and DTT (2.5-5 μl) or MnCl₂ (5 μl) and DTT (2.5-5 μl) separately)
 - 20-40 u RNasin ribonuclease inhibitor are recommended (when using low amounts of RNA, the addition of RNasin may be essential)
 - 1 μl (200 u) of M-MuLV RT
 - add nuclease-free water to 50 μl
 - mix by pipetting gently up and down
- Incubate the mixture at 37-55 °C for 30-120 min. The optimal time depends on the length of the cDNA: 30 min are recommended for 500 nt, 120 min for more than 1,500 nt length. The optimal temperature depends on the structural features of the RNA. Increase the temperature from 37 °C up to 55 °C for highly structured RNA. Note that optimal reaction time and temperature should be adjusted for each particular RNA.
- Heat the mixture to 90 °C for 10 min to inactivate the M-MuLV RT.

The cDNA (5-10 μl) can now be used as a template for amplification in the PCR. If the specific amplification requires the prior removal of the RNA add 2 u RNase H and incubate at 37 °C for 20 min.

Quality Control

Purified free of endo- and exodeoxyribonucleases, phosphatases and ribonuclease. Activity and stability tested in first strand cDNA synthesis.