The ENZYME Company

Reverase™ (M-MuLV RT) (M–MuLV Reverse transcriptase, RNase H minus)

Description *Reverase™* is M–MuLV Reverse transcriptase purified from *E.coli* strain harbouring a plasmid that directs the synthesis of modified form of *Moloney Murine Leukemia* virus (M-MuLV) reverse transcriptase. M-MuLV reverse transcriptase is an RNA or DNA directed DNA polymerase. The enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single stranded DNA as a template. This enzyme had been genetically altered to remove associated RNase H activity. Removal of the RNase H activity resulted in an increase of full-length cDNA products. MW of Reverase is 69 KDa

Concentration 50000-400000 units/ml

Storage buffer 50 mM TrisHCl, (pH 8,3), 100mM NaCl, 1mM EDTA, 0,1 mM DTT, 0,1%Triton X-100, 50% glycerol.

Recommended reaction buffer for RT-PCR (1X) 50 mM TrisHCl (pH 8,3 at 25°C), 2-8 mM $MgCl_2$, 10 mM DTT, 100 mM KCl (OPTIONAL: 2-4 mM $MnCl_2$,)

Supplied 5xRT buffer "complete" : 250mM TrisHCl (pH8,3), 500mM KCl, 15mM $MgCl_2$, 50mM DTT

Supplied 5xRT buffer "incomplete" : 250mM TrisHCl, pH8,3; 500mM KCl and extra tubes $MgCl_2$ (100mM) and DTT (100mM)

Unit definition One unit of activity is the amount of enzyme required to incorporate 1 nmole of dTTP into an acid-insoluble form in 10 minutes at 37°C using polyA-oligo(dT) as template and primer.

Storage condition: -20°C

cDNA synthesis with Reverase
Protocol

1. **Mix in the tube:**
   1-5 µg of the total RNA (or 50-500ng of polyA RNA)
   10 pmole of strand-specific primer (or 250-500ng of oligo-dT for each µg of RNA)
   add water up to 8 µl

2. **Incubate the mixture** 10 min at 70℃, then 10-15min at room temperature (for the specific primer) or place in ice in the case of oligodT or random primer

3. **Add into the mixture:**
   - 4 µl of **5xRT buffer complete** (250mM TrisHCl, pH8,3; 500mM KCl, 15mM MgCl2, 50mM DTT)
   - 1 µl of dNTP mix (10mM of each dNTP; Cat.-No: 110001 and 110002)
   - RNAsin – 20-40 units (optional)
   - Reverase – 200 units
   - H_2O – up to 20 µl

4. **Incubate the mixture** at 37-55℃ during 30 – 120 min. The time of reaction depends on the length of cDNA, 30 min if enough for cDNA in range of 500 bp in length, 120 min is for cDNA more then 1,5 kb. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 55℃) for the highly structured RNA. The optimal temperature and reaction time should be adjusted for each particular RNA. We recommend to use buffer with pH 8.8 if the reaction is performed at elevated temperature.

5. **Heat the mixture** 10 min at 65-70℃ to inactivate the Reverase.

6. **Use the mixture** for PCR or for other application.

Reverase and RTx5 buffer are free from RNase activities, meanwhile we recommend to add RNasin into the mixture to inhibit possible RNase contaminations of the sample.
For your PCR-Reaction you need approx. 5-10 µl of your RT-PCR product. Find the standard protocol for PCR in our Web-Page: PCR Theory Section.

Catalog #	Pack size
105100	10000 u
105250	50000 u

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