BIORON life science

Reverase™ (M-MuLV, RNase H minus)

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

Content

Ref No.	105100	105250	colour
Reverase™	10 000 units	50 000 units	blue
Incomplete RT * Reaction Buffer (5x)	1.0 mL	2x 1.0 mL	red
Complete RT ** Reaction Buffer (5x)	1.0 mL	2x 1.0 mL	yellow
$MgCl_2$ 100 mM	1.0 mL	2x 1.0 mL	green
DTT 100 mM	0.2 mL	2x 0.2 mL	white
Datasheet	1	1	--

* Incomplete RT Reaction Buffer (5x): 250 mM Tris-HCl pH 8.3, 500 mM KCl, free of $MgCl_2$ and DTT

** Complete RT Reaction Buffer (5x): 250 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 50 mM DTT

Application: First-strand synthesis of cDNA from RNA molecules. Reverase is used in cDNA synthesis with long templates. Resulted cDNA can be amplified by PCR with Taq polymerases. Reverase is suitable for detection of RNA viruses in combination with PCR amplification of cDNA.

Concentration: 200 – 500 units/µL (please see label)

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

Recommended reaction buffer for RT-PCR (1x): 50 mM Tris-HCl pH 8.3 at 25 °C, 2 – 8 mM $MgCl_2$, 10 mM DTT, 100 mM KCl (optional: 2 - 4 mM $MnCl_2$)

Quality Control

- 98 % protein homogeneity in 10 % SDS-PAGE
- First strand cDNA synthesis
- No detectable exo-/endonuclease and RNase activities
- PCR amplification tests with cDNA templates

Storage condition: -20 °C

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cDNA synthesis with Reverase™

Protocol

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

2. **Incubate the mixture** 10 min at 70 °C, then 10 – 15 min at room temperature (for the specific primer) or place on ice in case of oligo-dT or random primer.

3. **Add into the mixture:**
   - 4 µL of **5x RT buffer complete** (250 mM Tris-HCl pH 8.3; 500 mM KCl, 15 mM $MgCl_2$, 50 mM DTT)
   - 1 µL of dNTP mix (10 mM of each dNTP; Ref-No: 110001 and 110002)
   - RNase Inhibitor: 20 - 40 units (optional)
   - Reverase™: 200 units
   - Fill in H_2O to volume 20 µL

4. **Incubate the mixture** at 37 – 55 °C for 30 – 120 min. The time of reaction depends on the cDNA length, 30 min for cDNA in range of 500 bp length, 120 min for cDNA more than 1.5 kb. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 55 °C) for the highly structured RNA. The optimal temperature and reaction time should be adjusted for each particular RNA.

5. **Heat the mixture** 10 min at 65 – 70 °C to inactivate the Reverase™.

For further PCR amplification
Use the mixture for PCR or other applications. For your PCR-reaction you need approx. 5 – 10 µL of your RT-PCR product.

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