EasyScript ™ III RTase   (M-MLV RT RNsae H-)

Cat No: RTM103
Sise: 10000U   200U/ul

Description : EasyScript ™ III RTase, encoded by Moloney Murine Leukemia Virus (M-MLV RT) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template to which a primer has been hybridized. EasyScript ™ III RTase will also extend primers hybridized to single-stranded DNA. Second strand cDNA synthesis can be achieved from some RNA templates without an additional DNA polymerase EasyScript ™ III RTase can synthesized 9.5 kb products, the largest RNA component in the reaction. However, EasyScript ™ III RTase synthesized more Full-length cDNA regardless of size.

Reaction temperture: 50-55 °C

storage conditions: long time at -20 °C   short time at 4 °C

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.

Supplied 5x RT buffer : 500ul

250 mM TrisHCl, pH 8.3 , 375 mM KCl , 15 mM MgCl 2 , 50 mM DTT

Protocol

1. Mix in the tube: 0.1-5 µg of the total RNA (or 50-500 ng of polyA RNA) 5 pmole of strand-specific primer (or 250 to 500 ng of oligo-dT or random primer for each µg of RNA) add water up to 13 µl or to 14ul

2. Incubate the mixture 10 min at 70°C, stand on ice for 1 min and spin down.

3. Add into the mixture: 4 µl of 5x RT buffer
                             1 µl of dNTP mix 10mM
                             RNAsin – 20-40 units (optional)
                             1ul EasyScript ™ III RTase – 200 units
                             H_2O – up to 20 µl

4. Mix well and spin down the mixture, if using random primers incubation at 25°C for 5minutes.

5. Incubate the mixture at 50°C during 30-60 minutes. If necessary, can increase to 55 °C for difficult templates or specific gene primer.

5. Heat the mixture 15 min at 70°C to inactivate the RTase.

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

For your PCR-Reaction you need 1-10 µl of your RT-PCR product.