RT-0100

USER GUIDE: EBL MMLV Super RTase III, 10KU

Storage at -20°C

Description:

EBL MMLV Super RTase III, is an RNA-dependent DNA polymerase and with reduced RNase H activity and increase thermal stability. The Super RTase III can synthesize 9.5kb products and provide high specificity, high yields and more full-length cDNA.

Reaction temperture: 50-55˚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 5xRT buffer: 250 mM Tris-HCl, pH 8.3; 375 mM KCl; 15 mM $MgCl_2$; 50 mM DTT

Standard Protocol

1. Mix in the tube: 0.1-5 µg of the total RNA (or 50-500 ng of mRNA) and 5 pmole of strand-specific primer (or 250 to 500 ng of oligo -dT or 50-250 ng random primer for each µg of RNA) add nuclease-free water up to 13 or to 14 µl.
2. Incubate the mixture 10 min at 70°C, stand on ice for 1 minute and spin down
3. Set up each reaction as follows:

Component	Vol./reaction	Final concentration
5xRT buffer	4 µl	1x
dNTP mix, 10mM	1 µl	10mM/ 20µl reaction
RNase Inhibitors	X µl	20-40U (optional)
Super RTase III	1 µl	200U/ 20µl reaction

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.
6. Heat the mixture 15 min at 70°C to inactivate the RTase.
7. Use the mixture for PCR or for other application.