



Super RTase III

Cat No	Pack size	conc
RT0100	10000 U	200U/ul
RT0500	50000 U	200U/ul

Description:

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 fulllength cDNA.

Reaction temperture: 50-55°C

Storage conditions: -20°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 TrisHCl, pH 8.3
375 mM KCl
15 mM MgCl₂
50 mM DTT

Protocol

1. Mix in the tube: 0.1-5 μ g of the total RNA (or 50-500 ng of mRNA) 5 pmole of strand-specific primer (or 250 to 500 ng of oligo -dT or 50-250 ng random primer for each μ g of RNA) add water up to 13 or to 14 μ l
2. Incubate the mixture 10 min at 70°C, stand on ice for 1 minute and spin down
3. Add into the mixture:
4 μ l of 5xRT buffer
1 μ l of dNTP mix 10mM
RNAsin – 20-40 units (optional)
1ul Super RTase III– 200 units
H₂O – up to 20 μ l
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 μ l of your RT-PCR product.

ZymesetBiotek

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