# Super RTase III

**Cat. No.: 5060.02 50000U (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:

50-55ºC

## 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 $MgCl_2$
50 mM DTT

## Protocol:

1. **Mix in the tube:**
   0.1-5 µg 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 µg of RNA) add 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. **Add into the mixture:**
   4 µl of 5xRT buffer
   1 µl of dNTP mix 10mM
   RNAsin – 20-40 units (optional)
   1ul Super RTase III– 200 units
   $H_2O$– up to 20 µl

4. **Mix well and spin down the mixture**, if using random primers, incubation at 25ºC 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 applications.

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

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