Technogene

biology

# Technogene High efficient cDNA synthesis Kit

provides superior components that ensure efficient first-strand cDNA synthesis from mRNA, total RNA, Adenylated RNA templates.

The combination of highly efficient cDNA synthesis, effective RNase inhibition and pure dNTPs allows high yields of cDNAs of more than 13 kb.

cDNA synthesis Protocol:

- Thaw on ice and mix very well all reagents.
- Assemble and keep all reactions on ice.

- Combine the following in an RNase-free reaction tube

| | |
|---|---|
| 10X RT Buffer | 2 µL |
| 40mM dNTP (10mM of each) | 0.5 µL |
| Random Hexamer<br><br>Olido dT or specific primer | 1 µL<br>or<br>1 µL |
| RNA Template | 0.1–1 µg total RNA<br>or<br>50–500 ng mRNA (poly(A)) |
| PCR Grade Water(RNase/DNase free) | variable |
| RT Enzyme | 2 µL |
| Total valume | 20µL |

- Mix and collect the drops by centrifuging briefly.

Incubation:

1. Incubate 5 minutes at 65°C(to denature secondary structures)
2. .For Hexamer Primer, incubate 10 minutes at 25°C followed by 65°C for 30 minutes
   .For Oligo (dT) or gene-specific Primer incubate 1minute at 4°C and then incubate at 65°C for 30 minutes.

3. Inactivate enzyme at 80°C for 5 minutes.

Store products at –20°C or proceed to next step, such as PCR or qPCR.