Technogene biology

HyperTherm SynFusion
Reverse Transcriptase

Reverse Transcriptase is a modified, recombinant form of the Reverse Transcriptase from the Moloney Murine Leukemia Virus (M-MuLV)purified from *Escherichia.coli*

Reverse Transcriptase synthesizes a complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA (ssDNA) as a template

Features of Technogene HyperTherm SynFusion M-MuLV Reverse transcriptase include:

• High yields of full-length first strand cDNA up to 13 kb

• Optimum activity at 50–60°C

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

RT Enzyme	2 µL
10X RT Buffer	2 µL
DTT	0.8 µL
dNTP 40mM (10mM of each)	1 µL
Random Hexamer(10µM) Olido dT or specific primer(10µM)	1 µL or 1-2 µL
RNA Template	0.01–1 µg total RNA or 10–500 ng mRNA (poly(A))
PCR Grade Water(RNase/DNase free)	variable
Total valume	20µL

- Briefly spin tube to bring droplets to bottom and mix by pipetting.

Incubation:

- Hexamer Primer, incubate 10 minutes at 25°C followed by 52 °C for 10-30 minutes
- Oligo (dT) or gene-specific Primer incubate at 52 °C for 10-30 minutes.

- Inactivate enzyme at 80°C for 5 minutes.

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