



Product information

High Reverse Transcriptase Kit (RT-PCR)

Catalog #: 9K-005-0005
Size: 10,000U
Storage: -20°C

Description:

High RT Kit includes a recombinant genetically engineered version of a thermostable M-MULV reverse transcriptase. Since this is more thermostable than M-MLV, the synthesis of cDNA is possible over 50°C and this modified enzyme shows an optimal activity at 50°C. In addition, the synthesis of cDNA is achieved efficiently because 2nd structure of RNA can be retarded more easily comparing to that in low temperature. (below 42°C reaction)

Protocol

First-Strand synthesis of cDNA (20µL reaction volume)

1. Prepare the following mixture in a micro tube:

Template RNA	- Total RNA	10ng ~ 5µg
	- mRNA	1ng ~ 0.5µg
	- Specific RNA	0.01pg ~ 0.5µg
Primer	- oligo(dT) 20	50µM
	- Random hexamer	50µM
	- Sequencing specific	15 ~ 20pmol
	RNase free water	up to 10µL

2. Heat mixture to 65°C for 5 minutes and cool immediately on ice.

3. Add the following to the mixture IMMEDIATELY after cooling:

5X RT Reaction buffer	4µL
0.1M DTT	1µL
RNase Inhibitor	0.5µL
High RTase	1µL
RNase Free Water	up to 20µL

4. Mix gently on ice.

5. Incubate at 50°C for 50 min - using a PCR block or heating block instead of a water bath.

(Recommended to incubate at 37-42°C for 60 min in case of oligo dT 12-18 primer).

6. Inactivate the reaction by heating at 95°C for 5 minutes.

Advantages

- Thermostable and RNase H
- Synthesize cDNA at 42-50°C (peak activity at 50°C)
- Synthesis of long length cDNA (<14 kb)
- Synthesis primer : random primer, oligo-d(T), gene specific primer
- Excellent efficiency for synthesizing cDNA by inhibiting reducing secondary structure of RNA

Applications

- High-specific Long PCR
- High G+C and/or complex second structure DNA
- Amplification of low-copy DNA
- General sequencing
- TA cloning
- Difficult PCR

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Kit Contents:

- High RTase (200 U/ul).
- 5X High RT Reaction Buffer.
- RNase Inhibitor.
- 0.1M DTT.

Note:

Maintain storage temperature at -20°C
Use a [very stable heating source](#)
(PCR thermocycler recommended)
[Avoid water bath](#)