Product information

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℃ and this modified enzyme shows an optimal activity at 50℃. 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℃ 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
8mM 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.

High Reverse Transcriptase Kit

(RT-PCR)

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Advantages

- Thermostable and RNase H
- Synthesize cDNA at 42-50°C (peak activity at 50°C)
- Synthesis of long lenth 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

Kit Contents:

- High RTase (200 U/ul).
- 5X High RT Reac?on Buffer.
- RNAse Inhibitor.
- 8mM DTT.

Note:

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