cDNA Synthesis Kit

Catalog Number: NP041011610 – 50 reactions

Description:

cDNA Synthesis kit contains all necessary components for conversion of total RNA or mRNA to the single stranded cDNA. The 2X Buffer mix solutions contains, RT buffer, 1mM dNTP mixture, 8mM MgCl2, Oligo d(t)16, Random hexamer and stabilizer. Enzyme mix contains thermostable H-minus MMLV, RNase Inhibitor and stabilizer.

Features:

- Easy protocol
- Minimum pipetting steps
- RNase minus MMLV enzyme
- Long mRNA synthesis
- High temperature reaction to destabilize RNA secondary structures

Contents:

Components	
Buffer Mix (2X)	500 µl
Enzyme Mix	100 µl
DEPC-treated water	500 µl
cDNA Control Primer Mix (B2M)	50 µl

General Reaction Protocol (first strand cDNA synthesis):

1. Mix the template RNA (total RNA or Poly (A) mRNA) and other kit components in RNase-free tube as below table.
2. Mix the above mixture by quick vortex.
3. Incubate 10 min at 25°C.
4. Incubate 60 min at 47°C.
5. Stop the reaction by heating at 85°C for 5 minutes. Chill on the ice or at 4°C.

Component	Volume
Template RNA (1 ng to 5 µg)	X µL
Buffer-Mix (2x)	10 µL
Enzyme Mix	2 µL
PCR grade water	Up to 20µL final volume
Total Volume	**20 µL**

Note: To perform PCR, you can add the finished RT reaction up to 1:5 of the final PCR volume.

cDNA Control PCR Reaction:

Component	Volume
Taq 2X Premix	10 µL
cDNA Control primer mix	1 µL
cDNA	1 - 5 µL
PCR Grade Water	Up to 20 µL final volume
Total Volume	**20 µL**

1. Prepare a reaction mix according to the table.
2. For negative tube use PCR grade water. The final volume in each PCR reaction tubes is 20 µl.

Cycle	Time	Temp °C
1	4 min	95
35	30 sec	94
	30 sec	57
	30 sec	72
1	5 min	72

Note: It is recommended that all of the PCR components be premixed in a sufficient quantity for daily needs and then dispensed into the individual reaction tubes.

Agarose Gel Electrophoresis:

Run the total 5-7 µL of PCR products alongside 3 µL DNA marker on a 2% agarose gel containing Green Viewer Dye DNA safe stain.

The B2M primers amplify a band of approximately 230 bp from human, mouse and rat B2M cDNA.