KIAGENE FANAVAR

cDNA Reverse Transcription Kit

Cat. No:

FPLF012.0050
FPLF012.0100
FPLF012.0200

Contents:

Components	50 RXN	100 RXN	200 RXN
MMLV Reverse Transcriptase Enzyme aliquoted from Thermo Fisher co. (contain RNase Inhibitor)	100 µl	200 µl	400 µl
Positive Control primer	50 µl	100 µl	200 µl
2x Buffer (contain Oligo dt and Random hexamer	500 µl	1 ml	2 ml
RNase-Free Water	500 µl	1 ml	2 ml

Kit storage:

⚠ This kit should be stored at -20 °C.
If properly stored, all kit components are stable until the expiration date printed on the label.

Application

This kit provides a convenient and rapid method to produce single stranded cDNA from total RNA or mRNA and long mRNA. Kiagene cDNA Reverse Transcription Kit provides all the necessary components for cDNA synthesis except RNA template. The procedure is optimized to achieve reliable results within **90 min**.

Handling Requirements and Safety Information

⚠ *use RNase-free and DNase-free materials*

⚠ *Do not use any modified Protocols.*

⚠ *Do not pool reagents from different lot numbers.*

⚠ *Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.*

⚠ *After first opening store all bottles in an upright position.*

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

Protocol

To each sample

1. Add 1nanogram-5microgram template RNA (total RNA or mRNA) to a nuclease free tube.

2. Add 10µl 2x Buffer and 2µl reverse transcriptase Enzyme.

3. Add RNase-Free Water to reach 20µl totally. Vortex them quickly.

4. Incubate 10 min at 25 °C

5. Incubate 60 min at 47 °C

6. Incubate 5 min at 85 °C and store at 4°C

Checking the cDNA synthesis by positive control primer

1. Add 10µl 2x PCR Master Mix to a to a nuclease free tube.

2. Add 1µl Positive Control primer

3. Add 1-5µl cDNA.

4. Add RNase-Free Water to reach 20µl totally.

5. perform PCR with below program

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

6. Check 7 µl of PCR products by 0.5 % agarose gel electrophoresis. Store cDNA at 4˚C or -20˚C.
PCR product with Control primers should show a band with 230 bp from human, mouse and rat cDNA.