Easy™ cDNA Synthesis Kit

Description: *Easy cDNA Synthesis kit* contains all necessary components for conversion of total RNA or mRNA to ***the single stranded cDNA***. The 2 X RT-premix solutions contains MMLV RTase 200 units/10 µL, 100 mM Tris-HCl (pH 8.3), 20 mM DTT, 150 mM KCl, 2 mM dNTP mixture, 6 mM MgCl2, RNase Inhibitor 20 units, RTase Stabilizer and cDNA synthesis enhancers.

Component	A101161	A101162
RT-preMix (2x)	500 µL	1000 µL
Olio(dT)16 500 ng/µl	50 µL	100 µL
Random Hexamer 200 ng/µl	50 µL	100 µL
DEPC-treated water	500 µL	1.0 ml
cDNA con. Primer Mix (*B2M)*	50 µL	100 µL

Contents:

Kit storage:

This kit should be stored at -20°C. Unnecessary repeated freeze/ thawing should be avoided. Under these conditions reagents are stable for one year from the date of production.

General Reaction Protocol (first strand cDNA synthesis):

1- Mix the template RNA (total RNA or Poly(A)mRNA) and the primer in RNase-free tube as below table. Optimal reaction conditions, such as amount of RNA and primers, may vary and must be individually determined. Random hexamer or oligo (dT)16 or specific primers could be used as primer.

Concentration of template RNA and primer		
Template RNA	Total RNA or Poly(A)+mRNA	10 ng~5 µg or 5 ng~0.5 µg
Primer	Oligo (dT)16 or Random hexamer	1-2 µL or 1 µL
DEPC-treated water		Up to 10 µL

2- Incubate the mixture at 65 °C for 5 min and chill on ice.

3- Add 10 µL of RT Premix (2X).

4- Mix by pipetting gently up and down (total reaction volume 20 µL).

5- Incubate 10 min at 25 °C.

6. Incubate 60 min at 50 °C.

7. Stop the reaction by heating at 70 °C for 10 minutes. Chill on ice.

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	1x Rxn
10 x Buffer	2 µL
MgCl2 25mM	1.4
dNTP 10mM(2.5 Mm each)	1.2
cDNA Control primer mix	1 µL
cDNA	2
Taq DNA poly. (Not provided)	0.30 µL
PCR Grade Water	12.1
Final volume	20 µL

1- Prepare a reaction mix according to the table below.

2- For negative tube use 2µl of PCR grade water. The final volume in each PCR reaction tubes is 20µl.

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.

Amplification protocol

Cycle	Time	Temp °C
1	4 Min	95
	30 Sec	94
35	30 Sec	57
	30 Sec	72
1	5 Min	72

Agarose gel Electrophoresis

Run the total 7-10 µL of PCR products alongside 3µL DNA marker on a 2% agarose gel containing 0.5 µg/ml ethidium bromide.

The ***B2M*** primers amplify a band of approximately **320** bp from ***human B2M cDNA***.