

Easy™ cDNA Synthesis Kit

Cat: A101161 (50 Reactions)

Cat: A101162 (100 Reactions)

Store at -20 °C

Contents:

Component	A101161	A101162
<i>Buffer-Mix (2x)</i>	500 µL	1000 µL
<i>Enzyme Mix</i>	100 µL	200 µL
<i>DEPC-treated water</i>	500 µL	1000 µL
<i>cDNA Control Primer Mix (B2M)</i>	50 µL	100 µL

Description:

Easy 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

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.

<i>Template RNA</i>	1 ng~5 µg	XµL
<i>Buffer-Mix (2x)</i>		10
<i>Enzyme Mix</i>		2
<i>DEPC-treated water</i>		Up to 20 µL

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.

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

Component	Vol. (µL)
<i>Taq 2X Premix</i>	10
<i>cDNA Control primer mix</i>	1
<i>cDNA</i>	1-5
<i>PCR Grade Water</i>	Up to 20 µL

cDNA Control PCR Reaction:

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
	30 sec	94
35	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.

Amplification protocol:

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 DNA safe stain.

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

Disclaimers and Addresses:

This product is for **Research Use Only** and should only be used by trained professionals.

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