Parstous

# Easy cDNA Ultra-TM Synthesis Kit

(50 Reactions)
**Store at -20°C**

| Component | Volume |
|---|---|
| *Buffer-Mix (2x)* | 500 µL |
| *Ultra-Enzyme Mix* | 50 µL |
| *DEPC-treated water* | 500 µL |
| *cDNA Con. Primer Mix(B2M)* | 50 µL |

## Description

*Easy cDNA Ultra-TM Synthesis kit* contains all necessary components for conversion of total RNA or mRNA to ***the single stranded cDNA***. The 2X Buffer mix solution contains, RT buffer, 1Mm dNTP mixture, 8mM MgCl2, Oligo d(t)16, Random hexamer and stabilizer. Ultra-Enzyme mix contains **Ultra-TM thermostable H-minus MMLV** with an **optimum activity at 55 °C**, 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.

| | | |
|---|---|---|
| *Template RNA* | 1 ng~1 µg | 0.5-4 µL |
| *Buffer-Mix (2x)* | | 10 |
| *Ultra-Enzyme Mix* | | 1 |
| ***DEPC-treated water*** | | **Up to 20 µL** |

2- Mix the above mixture by quick vortex.
3- Incubate 10 min at 25 °C.
4. Incubate 30 min at **55 °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.

## cDNA Control PCR Reaction

1- Prepare a reaction mix according to the table.

| Component | Vol (µL) |
|---|---|
| *Taq 2X Premix* | 10 |
| *cDNA Control primer mix* | 1 |
| *cDNA* | 0.5-3 |
| *PCR Grade Water* | 7 |
| ***Final volume*** | **20 µL** |

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 |
| 35 | 30 Sec | 94 |
| | 30 Sec | 57 |
| | 30 Sec | 72 |
| 1 | 5 Min | 72 |

## Agarose gel Electrophoresis

Run the total 5-7 µL of PCR products alongside 3µL DNA marker on a 2% agarose gel containing Green viewer DNA safe stain.
The B2M primers amplify a band of approximately 230bp from human and mouse *B2M* cDNA.

### Disclaimers and Addresses

This product is for research use only and should only be used by trained professionals.

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