



UltraMarathonRT

20,000 U/mL | Store at -20°C

REVERSE TRANSCRIPTASE KIT

UltraMarathonRT is a group II intron encoded reverse transcriptase (RT) with ultra-high processivity and unmatched RNA structure unwinding power.

Components Provided

UltraMarathonRT Enzyme

2x UltraMarathonRT Buffer

UltraMarathonRT Boost

Nuclease-free Water

Components Not Provided

dNTP mix (10 mM each), Primer



Distributed by:

CliniSciences Group

First Strand cDNA Synthesis Quick Start Protocol



Above: uMRT Reverse Transcriptase Kit Components

ANNEALING RT PRIMERS TO RNA TEMPLATES

1. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

| Components | Volume (6 μ L total) |
|---|--------------------------|
| 1 pg–2 μ g of total cellular RNA or 1 pg– 500 ng of mRNA | 'x' μ L |
| Oligo-dT 18-30 primer (5 μ M) Random primer (10 μ M) Gene-specific primer (2 μ M) | 1 μ L |
| dNTP mix (10 mM each) | 1 μ L |
| Nuclease-free Water | (4-'x') μ L |

2. Incubate at 95°C for 30 sec and then snap cool on ice to anneal the primer to the template.

PREPARING THE RT REACTION MIXTURE

3. Add the components in a reaction tube as follows.

→ If RNA input is > 10 ng:

| Components | Volume (14 μ L total) |
|---------------------------------------|---------------------------|
| 2x UltraMarathonRT Buffer | 10 μ L |
| UltraMarathonRT Enzyme | 1 μ L |
| RNase Out™ (40 U/ μ L) (optional) | 1 μ L |
| Nuclease-free Water | 2 μ L |

→ If RNA input is \leq 10 ng, use UltraMarathonRT Boost:

| Components | Volume (14 μ L total) |
|---------------------------------------|---------------------------|
| UltraMarathonRT Boost | 1 μ L |
| UltraMarathonRT Buffer | 10 μ L |
| UltraMarathonRT Enzyme | 1 μ L |
| RNase Out™ (40 U/ μ L) (optional) | 1 μ L |
| Nuclease-free Water | 1 μ L |

4. Gently mix by tapping the tube and collect the liquid with a quick spin.
5. Add RT reaction mix to the annealed RNA and mix gently by tapping the tube.
6. Incubate at 42°C for 15 min for RNA \leq 12kb (or 1 hour for ultra-long RNA templates) to carry out reverse transcription.
7. Inactivate the enzyme by heating at 95°C for 1 min.
8. The cDNA can be used for subsequent analysis, such as PCR amplification, or stored at -20°C.

PCR AMPLIFICATION OPTIMIZATION

As a starting point, it is recommended to keep the volume of unpurified reverse transcription product (cDNA) 10% or less of the PCR reaction volume. For example, if the PCR reaction volume is 30 μL , use 3 μL or less unpurified reverse transcription product.

RT REACTION OPTIMIZATION

To improve cDNA yield, enzyme concentration may be increased to 60 units per reaction for a 20 μL reaction volume.

UltraMarathonRT performance is maintained at a temperature as low as 20°C, which avoids RNA degradation. Lower reaction temperatures require longer reaction times. Both the reaction time and temperature should be optimized according to the application.

STORAGE CONDITIONS

For short duration of storage (< 3 months), the enzyme can be placed in a -20°C freezer. For long-term storage, -80°C is recommended.

Note: UltraMarathonRT can endure 10 freeze-thaw cycles without losing noticeable activity. After 20 freeze-thaw cycles, UltraMarathonRT maintains 90% of its original activity.

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