

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

2x RT Reaction Buffer

High Boost

Nuclease-free water

Components Not Provided

dNTP mix (10 mM each), Primer



For product details, visit: www.RNAConnect.com

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), or Random primer (10 μ M), or 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 RT Reaction Buffer	10 μ L
UltraMarathonRT	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 High Boost:**

Components	Volume (14 μ L total)
High Boost	1 μ L
2x RT Reaction Buffer	10 μ L
UltraMarathonRT	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 30°C for 15 min to carry out reverse transcription. For RNA > 12 kb, 30°C for 20 - 60 min is recommended.
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 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 20 freeze-thaw cycles without losing noticeable activity. After 20 freeze-thaw cycles, UltraMarathonRT maintains 90% of its original activity.

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