RNAConnect

# UltraMarathonRT Reverse Transcription Kit

## A. INTRODUCTION

UltraMarathonRT (uMRT) is a highly optimized variant of MarathonRT with improved overall performance and sensitivity to low RNA input. uMRT is a group II intron encoded reverse transcriptase (RT) with ultra-high processivity and RNA template RNA unwinding capability. Unlike other RTs that require high temperature to disrupt stable structures, uMRT performance is maintained at ambient temperatures, from 20°C to 42°C, which prevents RNA template degradation.

## B. KITS COMPONENTS

| Part number | Description | Storage Recommendation |
|---|---|---|
| 200001 or 200002 | UltraMarathonRT (20 U/µL) | -20°C (up to 3mo)<br>-80°C (long-term)<br>Stable for 20x freeze/ thaw |
| 200011 | RT Reaction buffer (2x) | -20°C |
| 200012 | uMRT Boost (20x) | -20°C |
| 200013 | Nuclease-free water | Room temperature, 4°C, or -20°C |

## C. ENZYME INFORMATION

- **Unit definition:** One (1) unit is equal to the amount of UltraMarathonRT that incorporates 1 nmole dTTP at 42°C in 30 minutes, when using poly(rA) as the template and Oligo(dT)$_{18}$ as the primer.
- **Enzyme concentration and storage buffer:** The enzyme is supplied at 20 U/µL in a buffer that contains 25 mM K-HEPES pH 7.5, 300 mM KCl, 10% glycerol and 1 mM DTT.

## D. REACTION BUFFER (2X) COMPOSITION

Components and concentration in 2x:

- 100 mM Tris-HCl (pH 8.3 at 25°C)
- 400 mM KCl
- 8 mM MgCl2
- 10 mM DTT
- 40% glycerol

## E. ADDITIONAL REAGENTS REQUIRED AND NOT PROVIDED:

| Reagents | Recommendation |
|---|---|
| dNTP mix | Use a stock that contains 10mM each of dATP, dCTP, dGTP, and dTTP of high purity (>99%) in a pH 7.0 solution (e.g. New England Biolab, Cat# N0447) |
| Primers | Oligo-$(dT)_{18}$ primer (custom synthesis), random hexamer (e.g. Thermo Fisher, Cat# SO142) or gene-specific primer (custom synthesis) |
| RNA template | 1 pg–2 µg total RNA or 1 pg–500 ng of poly(A)-RNA. |

## F. STEP-BY-STEP PROCEDURE

### a. Anneal RT primers to RNA templates

- Combine the components as indicated in the table below to a nuclease-free microcentrifuge tube.
- Mix gently by tapping the tube. Collect the contents by brief centrifugation. Incubate at 95°C for 30 sec and snap cool on ice to anneal the primer to the template.

| Components | | Final amount | Volume |
|---|---|---|---|
| Primer | Oligo$(dT)_{18}$ (5 µM) | 5 pmol | 1 µL |
| | Randomer (10 µM) | 10 pmol | |
| | Gene-specific primer (2 µM) | 2 pmol | |
| Template RNA | Total RNA | 1 pg–2 µg | variable |
| | poly(A)-RNA | 1 pg–500 ng | variable |
| dNTP mix stock, 10 mM each | | 0.5 mM final concentration | 1 µL added to total |
| Nuclease-free water | | | 6 µL |

### b. Prepare the RT reaction mix

- Combine the components as indicated in the table below in a nuclease-free microcentrifuge tube.
- Mix the contents gently by tapping the tube and collect the contents by brief centrifugation.

| Components | Standard RNA Input (> 10 ng total RNA or PolyA RNA) | Low RNA Input (< 10 ng total RNA or PolyA RNA) |
|---|---|---|
| Nuclease-free water | 2 µL | 1 µL |
| Reaction Buffer (2x) | 10 µL | |
| UltraMarathonRT (20 U/µL) | 1 µL | |
| RNaseOUT™ (40 U/µL) (optional) (e.g. Thermo Fisher, Cat# 10777019) | 1 µL | |
| uMRT Boost (20x) | Not needed | 1 µL |
| Total volume | 14 µL | |

### c. Carry out the reverse transcription reaction

- Add the RT reaction mix (14 µL, prepared in Section **b**) to the annealed primer and RNA template (6 µL, prepared in Section **a**) to make a 20 µL reaction.
- Mix gently by tapping the tube.
- Incubate the mixture as indicated below.

| | For RNA ≤ 12 kb | For RNA ≥12 kb |
|---|---|---|
| cDNA Synthesis | 42°C for 15 min | 42°C for 60 min |
| Inactivation | 95°C for 1 min | |

The cDNA should be stored at -20°C until use. (**Note:** for PCR amplification, it is recommended to keep the volume of unpurified reverse transcription product (cDNA) input at 10% or less of the PCR reaction volume.)

## G. OPTIMIZATION AND TROUBLESHOOTING

| Potential Issue | Likely Cause | Solution |
|---|---|---|
| cDNA yields are low | Enzyme concentration is too low | Enzyme concentration may be increased up to 60 units per reaction for a 20 µL reaction volume |
| | Reaction time is too short | Consider extending the duration of the reaction – particularly if conducting it below 42°C |
| I did not get good results with my low abundant RNA samples | uMRT Boost was not utilized | For applications where the total amount of cellular input RNA is very low (10 ng or less), it is important to use uMRT Boost, an alternative uMRT buffer component that contains a specialized carrier molecule (RNAConnect catalog #R1003). |
| My PCR reaction did not work well | Too much uMRT Boost was used | uMRT Boost is only needed for very low RNA template input (< 10 ng).<br>High concentrations of uMRT Boost may inhibit some downstream PCR applications.<br>1 µL is the appropriate amount of uMRT Boost (20X) for a 20 µL reaction volume.<br>Clean up the cDNA samples before PCR reaction. |

## REFERENCES


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