RNAConnect

UltraMarathonRT® Two-Step RT-PCR Kit

A. INTRODUCTION

UltraMarathonRT (uMRT) is a group II intron encoded reverse transcriptase (RT) with ultra-high processivity (>30 kb templates in a single pass). Unlike other RTs that require high temperature to disrupt stable structures, uMRT has intrinsic helicase activity that actively unwinds RNA secondary and tertiary structures naturally without heat, preventing template degradation and allowing reverse transcription to occur at reaction temperatures ranging from 30°C, for any RNA template of any length or complexity. The PCR Master Mix contained in this kit is highly optimized for uMRT cDNA amplification of template lengths as long as 30 kb using RNA input as low as 0.1 pg. Combining the ultra-sensitivity and high performance of uMRT, this two-step RT-PCR kit provides an unmatched sensitivity to ultra-low RNA input, for applications with RNA templates of any length or structural composition.

B. KIT COMPONENTS

Description	Storage Recommendation	Part Number
UltraMarathonRT (20 U/µL)	-20°C (up to 3mo) -80°C (long-term) Stable for 20x freeze/thaw	200001, 200002
2x RT Buffer	-20°C	200003, 200009
High Boost	-20°C	200004, 200012
2x PCR Master Mix	-20°C	200050, 200051
dNTP mix	-20°C	200044, 200047
Oligo(dT)$_{18}$ Primer	-20°C	200045, 200048
Random Primer (15mer)	-20°C	200046, 200049
Nuclease-free water	Room temperature, 4°C, or -20°C	200013

C. UltraMarathonRT 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 condition:** The enzyme is supplied at 20 U/µL in a buffer that contains 25 mM K-HEPES pH 7.5, 300 mM KCl, 50% glycerol and 1 mM DTT. The enzyme can be stored in a -20°C non-frost-free freezer for up to 3 months. For long-term storage, -80°C is recommended.

D. PCR Master Mix

PCR Master Mix is a highly efficient 2x master mix for use in PCR applications. The master mix contains all the required components except for primers and template. The DNA polymerase included in this master mix has 80x higher fidelity than Taq and produces blunt-end PCR product. It is extremely processive and has robust efficiency in amplifying templates as long as 30 kb, even with high GC content.

E. ADDITIONAL REAGENTS REQUIRED AND NOT PROVIDED

Reagents	Recommendation
Gene-specific primers for PCR reaction	Custom synthesis
RNA template	0.1 pg – 2 µg total RNA with RIN > 8

F. STEP-BY-STEP PROCEDURE

a. Anneal RT primers to RNA templates

- Combine the components as indicated in the table below in a nuclease-free microcentrifuge tube.

Components		Final amount	Volume
Primer (Choose one)	Oligo(dT)$_{18}$ (5 µM), or	5 pmol	1 µL
	Randomer (15mer) (10 µM), or	10 pmol	
	Gene-specific primer (2 µM)	2 pmol	
Template RNA (Choose one)	Total RNA, or	0.1 pg – 2 µg	variable
	mRNA or specific template	0.1 pg – 500 ng	
dNTP mix (10 mM each)		0.5 mM each final concentration	1 µL
Nuclease-free water			added to total 6 µL

- Mix gently by tapping the tube. Collect the contents by brief centrifugation. Incubate at 95°C for 30 sec and then hold at 4°C in a PCR thermocycler.

b. Prepare the RT reaction mix

- Combine the components as indicated in the table below in a nuclease-free microcentrifuge tube on ice.

Components	Volume
Nuclease-free water	1 µL
2x RT Buffer	10 µL
UltraMarathonRT (20 U/µL)	1 µL
RNaseOUT® (40 U/µL) (optional – not included) (e.g. Thermo Fisher, Cat# 10777019)	1 µL
High Boost (optional)	1 µL
Total volume	14 µL

Note: If RNA input is ≤ 10 ng, High Boost is required

- Mix the contents gently by tapping the tube and collect the contents by brief centrifugation.

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 and collect the contents by brief centrifugation.
- Incubate the mixture as indicated below.

	For RNA ≤ 12 kb	For RNA ≥12 kb
cDNA Synthesis	30°C for 15 min	30°C for 20 – 60 min
Inactivation	95°C for 1 min	95°C for 1 min

Note: The cDNA can be stored at -20°C or immediately used for PCR amplification.

d. Amplify cDNA using PCR

- Combine the components as indicated in the table below on ice to prepare PCR reaction.

Components	Volume (µL)	Final Concentration
2x PCR Master Mix	25	1x
Forward primer (10 µM)	1.5	0.3 µM
Reverse primer (10 µM)	1.5	0.3 µM
Template DNA	1 - 10 µL of uMRT cDNA (step c)	
PCR Grade Water	12 – 21 µL	
Total	50 µL	

Note: Unpurified cDNA products generated by uMRT reverse transcription (steps a - c) can be used directly for PCR amplification. Up to 10 µL of the unpurified cDNA product can be used for a 50 µL PCR reaction. More than 10 µL of the unpurified cDNA may inhibit the PCR reaction.

PCR Cycling Conditions:

3-Step Cycle	Temp	Time
Denature	98˚C	10 sec
Anneal	(Tm - 5)˚C	15 sec
Extension	68˚C	10 sec / kb
(25-40 cycles)		

Note: If you're experiencing insufficient amplification, try any of the following alternatives:

- Increase primer concentration to a maximum of 0.5 µM.
- Increase the number of PCR cycles.
- Increase the extension time to 15 sec / kb.
- If the target transcript is > 10 kb, lower the primer concentration to a minimum of 0.15 µM and increase the extension time to 15 sec / kb.

G. OPTIMIZATION AND TROUBLESHOOTING

Potential Issue	Likely Cause	Solution
cDNA yields are low	uMRT concentration is too low	uMRT concentration may be increased up to 60 units per reaction for a 20 µL reaction volume
	RT Reaction time is too short	Consider extending the duration of the RT reaction, particularly if conducting it below 42°C
I did not get good results with my low abundant RNA samples	High 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 High Boost, an alternative uMRT buffer component that contains a specialized carrier molecule

REFERENCES

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