UltraMarathonRT®

20,000 U/mL | Store at -20°C

TWO-STEP RT-PCR KIT

This kit combines the ultra-high processivity and sensitivity of UltraMarathonRT with a carefully matched high fidelity DNA polymerase, allowing long RNA templates (up to 30 kb) to be efficiently amplified from ultra-low RNA inputs (as little as 0.1 pg).

Components Provided
UltraMarathonRT
2x RT Reaction Buffer
High Boost
dNTP Mix
Oligo $(dT)_{18}$ Primer
Random Primer (15 mer)
2x PCR Master Mix
Nuclease-Free Water

RNAConnect

RT-PCR Quick Start Protocol

ANNEALING RT PRIMERS TO RNA TEMPLATES

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

Components	Volume (6 µL total)
0.1 pg - 2 µg of total cellular RNA or 0.1 pg - 500 ng of mRNA	'x' µL
Oligo $(dT)_{18}$ (5 µM), or Randomer (15 mer) (10 µM), or Gene-specific primer (2 µM)	1 µL
dNTP Mix (10 mM each)	1 µL
Nuclease-Free Water	(4 - 'x') µL

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.

Components	Volume (14 µL total)
2x RT Reaction Buffer	10 µL
UltraMarathonRT	1 µL
RNase Out™ (40 U/µL) (optional)	1 µL
High Boost (optional)	1 µL
Nuclease-Free Water	Add to total 14 µL

➔ **If RNA input is ≤ 10 ng, High Boost is required.**

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 stored at -20 °C or immediately used for PCR amplification.

AMPLIFY THE cDNA USING PCR

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

Components	Volume (50 µL total)
2x PCR Master Mix	25 µL
Forward primer (10 µM)	1.5 µL
Reverse primer (10 µM)	1.5 µL
Template DNA	1 - 10 µL of uMRT cDNA (step 8)
PCR grade water	Add to total 50 µL

PCR Cycling		
3-StepCycle	Conditions Temp	Time
Denature	98 ˚C	10 sec
Anneal	(Tm - 5) ˚C	15 sec
Extension	68 ˚C	10 sec / kb

➔ **25 - 45 cycles**

PCR AMPLIFICATION OPTIMIZATION

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.

Up to 10 µL of the unpurified cDNA product (step 8) can be used for a 50 µL PCR reaction. More than 10 µL of the unpurified cDNA may inhibit the PCR reaction.

RT REACTION OPTIMIZATION

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

If low abundant RNA amplification is not consistently observed, make sure you are using High Boost.

STORAGE CONDITIONS

uMRT is stable in -20 °C for up to 3 months. For long-term storage, -80 °C is recommended. uMRT is stable for up to 20 freeze / thaw cycles.