UltraMarathonRT®

20,000 U/mL | Store at -20°C

TWO-STEP RT-qPCR KIT

This kit uses a green dye that is compatible with most of the real-time PCR systems available (Ex 498/Em 522), providing sensitive detection for any difficult RNA templates regardless of PCR amplicon location.

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

For product details, visit: www.RNAConnect.com

RT-qPCR 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. 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 (14 µL total)
2x RT Reaction Buffer Q	10 µL
UltraMarathonRT	1 µL
RNase Out™ (40 U/µL) (optional)	1 µL
Nuclease-Free Water	2 µL

4. Add RT reaction mix to the annealed RNA (step 2) and mix gently by tapping the tube.
5. Incubate at 30°C for 15 min to carry out reverse transcription. For RNA > 12 kb, 30°C for 20 - 60 min is recommended.
6. Inactivate the enzyme by heating at 95°C for 1 min.
7. The cDNA can be stored at -20°C or immediately used for PCR amplification.

AMPLIFY THE cDNA USING qPCR

8. Gently mix the following components to create a single qPCR reaction.

Components	Volume (20 µL total)
2x qPCR Green Dye Master Mix	10 µL
Forward primer (10 µM)	0.6 µL
Reverse primer (10 µM)	0.6 µL
Template DNA	1 - 2 µL of uMRT cDNA (step 7)
PCR grade water	Added to 20 µL

➔ Up to 2 µL of the unpurified cDNA product (step 7) can be used for a 20 µL qPCR reaction. More than 2 µL of the unpurified cDNA may inhibit the PCR reaction.

qPCR DATA COLLECTION:

Detection	Excitation Peak	Emission Peak
Green dye reagents	498 nm	522 nm

➔ Performing a melting curve analysis is important to evaluate the specificity of any dye-based qPCR assay. Specificity can also be confirmed using electrophoresis analysis on an agarose gel.

qPCR CYCLING CONDITIONS:

Stage of Cycle		Temp (˚C)	Time
Pre-Denaturation		98	1:30 min
Amplification (40-45 cycles)	Melt	98	10 sec
	Anneal	60	10 sec
	Extension[1]	68	30 sec
Melting Curve	Step 1	95	5 sec
	Step 2	65	1:00 min
	Step 3[2]	97	-
Cooling		40	10 sec

1. Be sure acquisition is set to ‘single’ at the Extension step. An extension time of 30 seconds is sufficient for all amplicon sizes ≤ 500 bp.
2. Be sure acquisition is set to ‘continuous’ with a ramp rate of 0.05 ˚C / sec at Step 3 of the Melting Curve cycle for smooth curves. Ramp rate for all other stages can be set to their maximum value.

→ For detailed qPCR troubleshooting suggestions, please view our full protocol available online (see QR code on cover).

STORAGE CONDITIONS

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

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