Fast MM Probe Multiplex OneStep RT-qPCR Kit

Fast MM Probe Multiplex OneStep RT-qPCR Kit is one-step RT-qPCR kit that provides accurate real-time quantification of RNA targets in gene expression analysis, using dual-labeled probes in easy to use master mix format. Master Mix (MM) contains all components for efficient RT-qPCR reaction with unique reverse transcriptase, highly processive hot start fast Taq DNA polymerase and ROX as reference dye.

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Kit Components

Component	Cat. No. E0830-01 100 reactions of 25 µl	Cat. No. E0830-02 500 reactions of 25 µl
2 x RT-qPCR Fast MM*	2 x 625 µl	10 x 625 µl
RNase-free Water	1.2 ml	5 x 1.2 ml

Storage:

All components of the kit should be stored at -20°C.

*Avoid multiple thawing and freezing (> 2) as this may reduce assay sensitivity. The reagent should be frozen in smaller aliquots if it is used only occasionally.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Description:

- 2 x RT-qPCR Fast MM is a universal solution for quantitative RT-qPCR one tube reaction using sequence-specific probes and can be used in most real-time PCR cyclers available.
- 2 x RT-qPCR Fast MM contains unique highly sensitive reverse transcriptase, fast Taq DNA polymerase and RNase inhibitor.
- Reverse transcriptase works in a high range of temperatures (35-55°C) without loss of specificity and sensitivity.
- Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA.
- Fast Taq DNA polymerase is a modified enzyme which provides very tight inhibition of the polymerase activity at moderate temperatures which is restored during the initial denaturation step at 95°C for 10 minutes.
- 2 x RT-qPCR Fast MM contains dNTPs and ROX dye. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument.

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Protocol:

Component	Volume/reaction	Final concentration
2 x RT-qPCR Fast MM	12.5 µl	1 x
Forward Primer	Variable	0.2-0.4 µM
Reverse Primer	Variable	0.2-0.4 µM
Probe	Variable	0.1-0.2 µM
Template RNA	Variable	1 pg-500 ng
RNase-free Water	To 25 µl	-
Total volume	25 µl	-

Notes:

1. Keep 2 x RT-qPCR Fast MM on ice, limit light exposure during handling to avoid loss of fluorescent signal intensity. Minimize thaw-freeze cycles of 2 x RT-qPCR Fast MM.
2. Thaw and gently mix by pipetting 2 x RT-qPCR Fast MM before use.
3. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
4. Optimal amplicon length in real-time RT-PCR using probes is 70-150 bp.
5. To avoid amplification from genomic DNA design exon-exon primers.
6. Set up RT-PCR reactions on ice to minimize RNA template degradation.
7. The RNA template (≤500 ng/reaction) should be added to the individual PCR tubes or wells containing the whole reaction mix. Centrifuge briefly before placing into cycler. Check if there are no bubbles left, if yes, spin again.
8. Place samples in the cycler and start the program.
9. Reverse transcriptase works in a wide range of temperatures (35-55°C). The recommended temperature for reverse transcription is 50°C. For the individual experiment temperature might be changed.
10. 2 x RT-qPCR Fast MM has optimized composition, there is no need to adjust any compound beside primers, probes and RNA concentration.
11. A final primer concentration of 0.4 µM is usually optimal, but can be individually optimized in the range of 0.1 µM to 1 µM. The recommended starting concentration is 0.4 µM. Raising primer concentration may increase PCR efficiency, but negatively affect RT-PCR specificity. Optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
12. Optimal melting temperature (T_m) of primers should be near 60°C. The T_m of dual-labeled probes should be 8-10°C higher than the T_m of the primers.
13. Readjust the threshold value for analysis of every run.
14. Avoid G at the 5'-end of the dual-labeled probe, which causes quenching of fluorescence signal.

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Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Reverse Transcription	50°C	10 min	1
Initial Denaturation	95°C	2 min	1
Denaturation Annealing/Extension/Data acquisition	95°C 60°C	5 s 30 s	40-45
Cooling	4°C	Indefinite	1

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