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Probe Multiplex OneStep RT-qPCR Kit

Kit Components:

Probe 1-Step RT-qPCR Kit (2x)

Component	Cat. No. E0819-01 100 reactions, 25 µl each, 2.5 ml [1x] final volume	Cat. No. E0819-02 200 reactions, 25 µl each, 5 ml [1x] final volume
[4x] Buffer Mix	650 µl	5 x 650 µl
Enzyme Mix	100 µl	500 µl
Water, nuclease free	1500 µl	5 x 1500 µl

Probe 1-Step RT-qPCR Kit (2x) plus ROX Solution

Component	Cat. No. E0819-03 100 reactions, 25 µl each, 2.5 ml [1x] final volume	Cat. No. E0819-04 200 reactions, 25 µl each, 5 ml [1x] final volume
ROX solution, 25 µM	15 µl	60 µl
[4x] Buffer Mix	650 µl	5 x 650 µl
Enzyme Mix	100 µl	500 µl
Water, nuclease free	1500 µl	5 x 1500 µl

Storage:
Store at -20˚C in the dark.
Avoid repeated thawing and freezing (more than four times), as this may reduce assay sensitivity. Freeze the component in aliquots if they are only used intermittently.

Probe Multiplex OneStep RT-qPCR kit is a one-step RT-qPCR kit providing accurate real-time quantification of RNA targets in gene expression analysis, using dual-labeled probes. The kit contains a unique, sensitive, qPCR-optimized reverse transcriptase and highly processive hot start TiTaq DNA Polymerase, combined in an easy-to-use kit format.

Description:

- 4 x Buffer Mix is a universal solution for quantitative RT-qPCR one tube reaction using sequence-specific probes and can be used on most real-time PCR cyclers available.
- The Enzyme Mix contains unique highly sensitive reverse transcriptase, hot start tiTaq DNA Polymerase, and RNase Inhibitor.
- Reverse transcriptase works in a high range of temperatures from 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.
- tiTaq DNA Polymerase is a modified "hot start" 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 2-5 minutes.
- 4 x Buffer Mix contains dNTPs.
- The kit has been designed while keeping short qPCR run times in mind.

Probe Multiplex 1-Step RT-qPCR Kit

REAL TIME PCR PROTOCOL (1)

qPCR- Protocol

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 25 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.5 µl	500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.5 µl 10 x diluted (in water)	50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

Preparation of qPCR Reaction:

Component	Volume/Reaction	Final Concentration
[4x] Buffer Mix	6.5 µl	1x 4 mM $MgCl_2$
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
Optional: ROX Solution, 25 µM	0.5 µl or 0.5 µl 10 x diluted	500 nM 50 nM
Enzyme Mix	1 µl	1 µl / reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. **Keep Dark:** Keep Probe Enzyme Mix and ROX solution on ice, limit light exposure during handling to avoid loss of fluorescent signal intensity. Minimize freeze-thaw cycles of [4x] Buffer Mix. Thaw and gently mix by pipetting [4x] Buffer Mix before use.
2. **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.
3. **Recommended Amplicon Length:** The optimal amplicon length in real-time Probe qRT-PCR is 70-150 bp.
4. **Avoid Concentration Differences:** Thaw and mix [4x] Buffer Mix by gentle pipetting.
5. **Set Up On Ice:** Set up RT-PCR reactions on wet ice to minimize RNA template degradation.
6. **Use Exon - Exon Primers**, if possible, to avoid amplification from genomic DNA.
7. **Assay Preparation:** Add RNA template (recommended 500 ng/reaction, although much less material might be required) to the individual PCR tubes or wells containing the whole reaction mix. Centrifuge briefly before placing the assays into the cycler. Check if there are no bubbles left. In case of bubble leftovers, spin again to remove all remaining bubbles.
8. **Start qPCR:** Place the assays in the cycler and start the program.
9. **Template DNA Amount:** Reverse transcriptase works in a broad temperature range between 35°C and 55°C. The recommended temperature for reverse transcription is 50°C. Reaction temperature may be varied accordingly to meet specific assay requirements.
10. **$MgCl_2$ Concentration:** Standard concentration of $MgCl_2$ in real-time RT-PCR reaction is 4 mM (as provided with the 1x Buffer Mix). In most cases this concentration will produce optimal results. However, if higher $MgCl_2$ concentrations are required, prepare a 25 mM $MgCl_2$ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 25 µl will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 1.0 mM.
11. **Primer Concentration:** 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. **Melting Temperature:** Optimal melting temperature (Tm) of primers should be near 60°C. The Tm of dual-labeled probes should be 8-10°C higher than the Tm of the primers.
13. **Threshold Value:** Readjust the threshold value for analysis of every run.
14. **Probe Design:** Avoid G at the 5'-end of the dual-labeled probe, which causes quenching of the fluorescence signal.

Probe Multiplex 1-Step RT-qPCR Kit

REALTIME PCR PROTOCOL (2)

qPCR- Protocol - Thermal Cycling Conditions

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Reverse Transcription	50˚C	15 min	1
Initial Denaturation	95˚C	2 min	1
Denaturation	95˚C	10 s	40-45
Annealing / Extension / Data acquisition	60˚C	40-60 s	
Cooling	4˚C	Infinite	1

Notes:

1. **Quality Control - Agarose Gel Check:** When designing a new assay, always check the PCR product specificity by gel electrophoresis. Caution: Melting temperatures of the specific PCR product and of undesired primer-dimers may overlap.