EURx® MOLECULAR BIOLOGY PRODUCTS

Probe OneStep RT-qPCR kit

Probe 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. Kit is composed of unique reverse transcriptase and highly processive hot start onTaq DNA Polymerase in easy to use format.

Probe OneStep RT-qPCR kit

Kit Components

Component	Cat. No. E0812-01 25 reactions of 25 µl	Cat. No. E0812-02 100 reactions of 25 µl
2 x RT-qPCR Probe Buffer	1 x 350 µl	2 x 0.7 ml
Probe Enzyme Mix	25 µl	100 µl
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 0.5 ml	2 x 1 ml

Probe OneStep RT-qPCR kit, plus ROX Solution

Kit Components

Component	Cat. No. E0813-01 25 reactions of 25 µl	Cat. No. E0813-02 100 reactions of 25 µl
2 x RT-qPCR Probe Buffer	1 x 350 µl	2 x 0.7 ml
ROX Solution, 25 µM	15 µl	60 µl
Probe Enzyme Mix	25 µl	100 µl
Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	10 µl	30 µl
Water, nuclease free	1 x 0.5 ml	2 x 1 ml

Storage

Store at -20°C in the dark.

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

The kit provides:

- 2 x RT-qPCR Probe Buffer 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 Probe Enzyme Mix contains unique highly sensitive reverse transcriptase, hot start onTaq 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.
- onTaq 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 at least ten minutes.
- 2 x RT-qPCR Probe Buffer contains dUTP, which partially replaces dTTP. It allows the optional use of thermolabile uracil -N-glycosylase (UNG) to prevent carryover contamination between reactions. UNG removes uracil from any dU-containing contaminating amplicons at moderate temperatures and is inacivated during RT step at 50°C.
- There are two variants of the kit: without ROX and with ROX Solution provided separately. 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. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.

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, ViiA 7, 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	-

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

Protocol

Component	Volume/reaction	Final concentration
2 x RT-qPCR Probe Buffer	12.5 µl	1 x
Forward Primer	Variable	0.4 µM
Reverse Primer	Variable	0.4 µM
Probe	Variable	0.1-0.2 µM
Template RNA	Variable	1pg-500 ng
Optional: ROX Solution, 25 µM	0.5 µl or 0.5 µl 10 x diluted	500 nM 50 nM
Probe Enzyme Mix	1 µl	1 µl /reaction
Optional: Thermolabile UNG (uracil-N-glycosylase) 1 U/µl	0.25 µl	0.25 U/reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. Keep Probe Enzyme Mix and ROX solution on ice, limit light exposure during handling to avoid loss of fluorescent signal intensity. Minimize thaw-freeze cycles of 2 x RT-qPCR Probe Buffer.
2. Thaw and gently vortex 2 x RT-qPCR Probe Buffer 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 the 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. Standard concentration of $MgCl_2$ in real-time RT-PCR reaction is 3 mM (as provided with the 1 x RT-qPCR Probe Buffer in most cases this concentration will produce optimal results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution and add to the reaction.
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.

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

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Reverse Transcription	50°C	20 min	1
Initial Denaturation	95°C	15 min	1
Denaturation Annealing/Extension/Data acquisition	94°C 60°C	15 s 60 s	40-45
Cooling	4°C	Indefinite	1

Notes:

1. During reverse transcription step of 50°C also thermolabile uracil-N-glycosylase might be used. Do not use UNG from *E.coli*, UNG will degrade all newly synthetized cDNA.
2. Always check the RT-PCR product specificity by gel electrophoresis when designing a new assay. Melting temperatures of the specific product and primer-dimers may overlap.

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