•GeneON•

DATASHEET

One.step RT-qPCR Kit *"Perfect"* (5X)

Cat: 106-100, 200 rcs.

For quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes

Description

The One.step RT-qPCR Kit (5X) is designed for quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes. The enzyme mix is based on a with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments.

The kit contains all reagents required for RT-qPCR (except template, primers and the dual labeled fluorescent probe) in one box to ensure fast and easy preparation with a minimum of pipetting steps. The premium quality enzyme mixes **(5x PCR Mix and 20x RT Mix)** and the optimized complete reaction buffer containing ultrapure dNTPs ensure superior real time PCR results.

RT-qPCR is used to amplify double-stranded DNA from single-stranded RNA templates to allow a rapid real-time quantification of RNA targets. In the reverse transcription step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template.

In the first cycle of the PCR step the hot-start DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. The hot-start polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.

One-step RT-qPCR offers tremendous convenience when applied to analysis of targets from multiple samples of RNA and minimizes the risk of contaminations.

Benefits:

- High sensitivity
- Reverse transcription and real time PCR setup in one-step
- includes optimized RNase Inhibitor Reverse Transcriptase mixture
- Low risk of contamination and pipetting error
- Compatible with all standard real-time PCR cyclers

Platforms: The Kit is suitable for all block-based Thermocycler.

30 nM ROX:
Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.

300 nM ROX final concentration:
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.

Components:

1 Tube with 5X PCR complete-Mix;
1 Tube with 20X RT complete-Mix;
1 Tube with ROX 200µM for optional use

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RT-PCR assay without sample denaturation (standard RNA/primer combinations)

Considerations pre protocol:

Please note to protect RNA from degradation:
- prepare RNA samples in a separate laboratory area not used for setting up reactions;
- use nuclease free gloves and labware;
- prevent the RNA degradation by spreading RNases;
- check the quality of the RNA sample on a denaturing agarose gel prior to cDNA synthesis;

Preparation of the RT-PCR Assay:

Please note: Sample denaturation is particularly recommended for RNA targets that exhibit a high degree of secondary structure, for self- or cross-complementary primers and for initial experiments with new targets. For many standard combinations of RNA and primers heat treatment may be omitted with no negative effect on results. Add the following components to a nuclease-free micro-tube. Pipette on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the rest of the components are added.

component	stock conc.	final conc.	20 µl assay	25 µl assay
Specific Probe		200 nM		
Total RNA or mRNA Template[1)]		1 pg - 1 µg or > 0,01 pg	X µl	X µl
forward Primer		100 - 400 nM		
reverse Primer		100 - 400 nM		
20X RT complete-Mix	20X	1-2**	1-2 µl	1,25-2,5 µl
One.step RT-qPCR Kit (5X)	5X	1x	4 µl	5 µl
ROX 1:100 *** ROX 1:10 ***		30 nM - LOW ROX * 300 nM - HIGH ROX *	0,3 µl 0,3 µl	0,4 µl 0,4 µl
PCR-grade Water			up to 20 µl	up to 25 µl

*) Optional, depending on Real-time PCR instrument

**) Increased amounts of RT-Mix (up to 2x) improve number of cycles values, but primer dimers might appear

***) If needed, prepare a fresh dilution of ROX internal reference dye. For a final reaction concentration of 30 nM dilute 200 µM ROX 1:100 in PCR grade water. For a final reaction concentration of 300 nM dilute 200 µM ROX 1:10 in PCR grade water. For a final reaction volume of 20 µl add 0.3 µl of the ROX dilution. The diluted ROX reference dye must be kept in a light-protected tube at 4 °C.

Note: We recommend an amplicon size of less than 220 base pairs

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Mix gently and run your RT-Cycler program

Thermal cycling Place the vials in a PCR cycler and start the following program.

reverse transcription [3]	50 - 55°C	10-11 min	1x
initial denaturation [4]	95°C	12-15 min	1x
denaturation	95°C	5 - 15 sec	Up to 45 x
annealing and elongation	60-65°C [5]	30 - 60 sec. [6]	Up to 45 x

[3] A reverse transcription time of at least 10 min is recommended for optimal amplicon lengths between 100 and 200 bp. For longer amplicons prolong the incubation time of 15 min. Add 3 min for each additional 100 bp. The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.

[4] An initial denaturation time of at least 12 min is recommended to inactivate the reverse transcriptase

[5] The annealing temperature depends on the melting temperature of the primers and DNA probe used.

[6] The elongation time depends on the length of the amplicon.

Storage:
@ -20°C, avoid frequent thawing and freezing

Transport:
The product will be shipped with "blue ice"

Ordering information

Cat.-no	Description	Amount
106-100	One.step RT-qPCR Kit “perfect” (5X)	200 rcs / 20µl
106-100L	One.step RT-qPCR Kit “perfect” (5X)	2000 rcs / 20µl
106-100XL	One.step RT-qPCR Kit “perfect” (5X)	10000 rcs / 20 µl

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary. Note that optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.

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