Dye-based one-step RT-qPCR kit with high carboxy-X-rhodamine (100 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22110-100

www.zellx.de

Detection and quantification of DNA and cDNA targets, Gene expression using standard and fast qPCR platforms

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Introduction

ZellX® One-Step Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) Kit grants efficient cDNA synthesis and quantitative/real-time PCR in a single tube. The PCR Master Mix provided in the kit contains a green fluorescent reporter dye plus all the reagents (except primers and RNA template) needed for running real-time PCR reactions. In addition, a separate Reverse Transcriptase mix that comprises a balanced mixture of both Reverse Transcriptase and RNase Inhibitor is provided. As an internal reference, the kit contains high concentrations of carboxy-X-rhodamine (ROX™). Being independent from the amount of DNA template, the fluorescence signal of ROX™ is not influenced by the PCR reactions, and therefore can assist in the normalization of the reporter-dye signal during data analysis. The appropriate level of ROX™ depends on the real-time PCR instrument (Contact your instrument manufacturer for details). For low levels of ROX™ use our **Dye-based one-step RT-qPCR kit with low carboxy-X-rhodamine (Cat NO. ZX-22109-100/200/400)**.

RT-PCR is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR, Taq DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. During subsequent cycles, the DNA polymerase exponentially amplifies the double-stranded DNA template.

Materials supplied in the Kit

Component	*Quantity*
qPCR Master Mix (2X)	1 mL
Reverse Transcriptase mix	200 µL
RNase free water	1 mL

Storage instruction

All reagents should be stored at -20°C upon receipt. Avoid repeated freezing and thawing.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips (RNase & DNase free)

Nuclease-free tubes / strips / plates corresponding to the PCR device

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed. PCR machine / Thermocycler must be turned on and programmed in advance to avoid delays after setting up the reactions. (Do not forget to enable the option to record ROX™ fluorescence as the passive dye).
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay Procedure

For 20 µL reaction

- Thaw all kit components on ice and mix them well. Collect liquid at the bottom of the tube with a quick spin.
- Set up the following reaction mixture.

Component	*Quantity*
qPCR Master Mix	10 µL
Reverse Transcriptase mix	2 µL
Forward Primer	1-2 µL (100-400 nM)
Reverse Primer	1-2 µL (100-400 nM)
RNA template	0.01 pg-1 µg*
Nuclease-Free Water	Up to 20 µL

**For optimal performance, we recommend to use 1 pg-1 µg total RNA or > 0.01 pg mRNA*

- Mix reagents thoroughly, and transfer to the thermocycler.
- Run the appropriate PCR cycling protocol on your real-time PCR instrument

Step	*Number of Cycles*	*Temperature*	*Duration*
Reverse transcription	1	42°C	30 min
Initial activation	1	95°C	3 min
Amplification*	40	95°C	5 sec
		60-65°C*	20-30 sec

**Not < 60°C.*

- The appropriate PCR cycling protocol must be optimized by the end user
- high primer concentrations result in nonspecific amplification and should be avoided