



**Probe-based one-step RT-qPCR kit  
with high carboxy-X-rhodamine  
(400 rxns)**

Zellbio GmbH (Germany)

CAT No. ZX-22108-400

[www.zellx.de](http://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

The 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 all the reagents (except primers, probes and RNA template) needed for running real-time PCR reactions and is compatible with different probe technologies (including Taqman®, Molecular Beacons® and Scorpion® probes). 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 **Probe-based one-step RT-qPCR kit with low carboxy-X-rhodamine (Cat NO. ZX-22107-100/200/400)**.

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

<i>Component</i>	<i>Quantity</i>
qPCR Master Mix (2X)	4 mL
Reverse Transcriptase mix	800 µL
RNase free water	4 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.

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

<i>Step</i>	<i>Number of Cycles</i>	<i>Temperature</i>	<i>Duration</i>
<b>Reverse transcription</b>	1	42°C	30 min
<b>Initial activation</b>	1	95°C	3 min
<b>Amplification*</b>	40-45	95°C	15 sec
		60-68°C*	30 sec

*\* Not < 60°C. Per our recommendation 2°C above the melting temperature (TM) of primers.*

- The appropriate PCR cycling protocol must be optimized by the end user.
- Use a primer with melting temperature (TM) around 60 °C.
- Use maximum 400 bp amplicons for standard qPCR and between 80 bp and 200 bp for fast qPCR.
- In case of using TaqMan® probes, choose probe near to 5' primer, and avoid terminal guanosine residues.