Probe-based qPCR Master Mix (high cxr)

(250 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22115-250

www.zellx.de

Post reverse transcription step detection and quantification of DNA and cDNA targets, low copy gene detection, Gene expression using standard and fast qPCR platforms

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

Table of Contents

Please read this insert completely prior to using the product.

Introduction

ZellX® Probe -based qPCR Master Mix (high cxr) grants efficient quantitative/real-time PCR in a single tube. The probe-based qPCR Master Mix contains all the reagents (except primers, probes and DNA template) needed for running real-time PCR reactions and is specially designed for Taqman® probe technologies (Based on the TaqMan® probe detection principle, the reporter dye (at 5' end) and quencher (at 3' end) hybridize on a specific region within the amplified fragment (Probe-Target Binding site). Amplification of the target gene results in cleavage of probe and release of the reporter dye (fluorophore). The intensity of detected fluorescent signal is proportional to the number of amplicons).

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 qPCR Master Mix (low cxr) (Cat NO. ZX-22114-250/500/1000)**.

Materials supplied in the Kit

Component	*Quantity*
qPCR Master Mix (2X)	2 x 1.25 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 Master Mix 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
Forward Primer	X µL (200 nM)
Reverse Primer	X µL (200 nM)
TagMan Probe	100-300 nM
DNA template	10-100 ng
Nuclease-Free Water	Up to 20 µL

**For optimal performance, we recommend to use cDNA corresponding to 1 pg to 500 ng of total RNA. For genomic DNA, we recommend to not use more than 100 ng*

- 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*
Initial activation	1	95°C	30 sec
Amplification*	40	95°C	15 sec
		TM (60-68°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