# Fluorescent Green qPCR Master Mix (low cxr)

# (1000 rxns)

ZELLX GmbH (Germany)

CAT No. ZX-22112-1000

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 !!!**

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Please read this insert completely prior to using the product.

## Introduction

ZellX® Fluorescent Green qPCR Master Mix (low cxr) grants efficient quantitative/real-time PCR in a single tube. The Fluorescent Green qPCR Master Mix contains a green fluorescent reporter dye plus all the reagents (except primers and DNA template) needed for running real-time PCR reactions. As an internal reference, the kit contains low 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 high levels of ROX™ use our **Fluorescent Green qPCR Master Mix (high cxr) (Cat NO. ZX-22113-500/1000)**.

## Materials supplied in the Kit

| *Component* | *Quantity* |
|---|---|
| **qPCR Master Mix (2X)** | 8 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) |
| **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 | 3-5 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