# One-step RT-PCR kit

# (400 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22106-400

www.zellx.de

**Suitable for Amplification of GC-rich and complex templates**

**!!! 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 Reverse Transcription Polymerase Chain Reaction (RT-PCR) Kit grants efficient cDNA synthesis and PCR in a single tube. The PCR Master Mix provided in the kit contains all the reagents (except primers and RNA template) needed for running standard PCR reactions. In addition, a separate Reverse Transcriptase mix that comprises a balanced mixture of both Reverse Transcriptase and RNase Inhibitor is provided

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* |
|---|---|
| **PCR Master Mix (2X)** | 10 mL |
| **Reverse Transcriptase mix** | 1 mL |
| **RNase free water** | 8 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.

- 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 50 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* |
|---|---|
| **PCR Master Mix** | 25 µL |
| **Reverse Transcriptase mix** | 2.5 µL |
| **Forward Primer** | 2 µL (0.4 µM) |
| **Reverse Primer** | 2 µL (0.4 µM) |
| **RNA template** | 0.1–1 µg* |
| **Nuclease-Free Water** | Up to 50 µL |

**For optimal performance, we recommend to use 0.1–1 µg total RNA or 10–500 ng mRNA*

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

| *Step* | *Number of Cycles* | *Temperature* | *Duration* |
|---|---|---|---|
| **Reverse transcription** | 1 | 42°C | 30 min |
| **Initial activation** | 1 | 95°C | 3 min |
| **Amplification*** | 35-40 | 95°C | 15 sec |
| | | 55°C* | 30 sec |
| | | 72°C | 30-60 sec/kb |
| **Extension** | 1 | 72°C | 5 min |
| **cooling** | 1 | 4°C | ∞ |

**approximately 5°C below the melting temperature (TM) of primers*

- The appropriate PCR cycling protocol must be optimized by the end user