One-step RT-PCR kit

(100 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22106-100

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 !!!

Table of Contents

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)	2.5 mL
Reverse Transcriptase mix	250 µL
RNase free water	2 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