A&A BIOTECHNOLOGY
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Manual

TranScriba™ 1step PCR Mix Sybr®

Kit for reverse transcription followed by real-time PCR with Sybr® Green.

catalog #	size
2008-100S	100 reactions in 25 µl
2008-200S	200 reactions in 25 µl

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Description

TranScriba™ 1step PCR Mix Sybr® is ready to use reverse mixture for reverse transcription followed by real-time PCR with Sybr® Green. TranScriba™ reverse transcriptase is a MMLV-based reverse transcriptase especially formulated for qPCR. TranScriba™ reverse transcriptase optimal activity is achieved at 50 °C, when it's used in one-step qRT-PCR with 1step PCR Mix Sybr®.

Mixture contains all components except DNA template and primers.

The premix formulation saves time and reduces contamination due to a reduced number of pipetting steps required for qPCR set up. The mix is optimized for efficient and reproducible reaction one-step qRT-PCR.

Contents

	2008-100S	**2008-200S**	**storage**
1step PCR Mix Sybr®	1.25 ml	2 x 1.25 ml	-20 °C
TranScriba™ reverse transcriptase	50 µl	100 µl	-20 °C
RNAse inhibitor	60 µl	60 µl	-20 °C
DTT	50 µl	50 µl	-20 °C
sterile water	1.5 ml	2 x 1.5 ml	-20 °C

1step PCR Mix Sybr® composition

component
***Taq* DNA polymerase**
$MgCl_2$
dNTPs
2x reaction buffer with Sybr®Green

Notes

- Before use all solutions should be thoroughly thawed and mixed by inverting the tube.
- Use certified nuclease-free labware.
- Work sterile and use all RNA lab work precautions, wear gloves and change them whenever appropriate.
- Up to 7x repeated freeze-thaw cycles do not influence the activity of this product, but may cause a slight decrease in fluorescence.

ROX reference dye

Some PCR instruments perform fluorescence signal correction and it is recommended to use ROX reference dye for signal normalization. Please follow manufacturer's instructions regarding addition of ROX reference dye and its concentration.

Example PCR protocol

1. Thaw **the all components** on ice, gently mix and briefly centrifuge. Place the tubes on ice again.
2. Place a sterile PCR tube on ice and add:

	PCR reaction volume		
component	**10 µl**	**20 µl**	**25 µl**
1step PCR Mix Sybr®	5 µl	10 µl	12,5 µl
RNAse inhibitor	0.1 µl	0.2 µl	0.25 µl
primer 1**	0.05-0.5 µM*	0.1-1 µM*	0.1-1 µM*
primer 2**	0.05-0.5 µM*	0.1-1 µM*	0.1-1 µM*
DTT	0.1 µl	0.2 µl	0.25 µl
TranScriba™ reverse transcriptase	0.2 µl	0.4 µl	0.5 µl
RNA or mRNA template	0.1 pg -100 ng RNA 0.1 pg - 1 ng mRNA	0.1 pg -100 ng RNA 0.1 pg - 1 ng mRNA	0.1 pg -100 ng RNA 0.1 pg - 1 ng mRNA
sterile water	up to 10 µl	up to 20 µl	up to 25 µl

* recommended for standard qPCR
** final concentration in reaction mixture

3. Gently vortex the samples and briefly centrifuge.
4. Place the tubes in the thermocycler and start the qRT-PCR programme.

Note: Specific RT-PCR conditions are to be optimized for each amplicon. Difficult templates (GC-rich RNA or secondary structure RNA) generally require longer denaturation and annealing/extension times.

step	**temperature**	**time**
reverse transcription	50 °C	10 min
initial denaturation	95 °C	3 min
40 cycles	95 °C 58-70 °C*	15-30 s 15-60 s**

* depending on the elongation of primers temperature
** depending on the length of PCR products and/or number of amplicons in the tube

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