

Manual

TranScriba™ 1step PCR Mix Probe

Kit for reverse transcription followed by real-time PCR with fluorescent probes.

catalog #	size
2008-100Q	100 reactions in 25 µl
2008-200Q	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 Probe is ready to use reverse mixture for reverse transcription followed by real-time PCR with fluorescent probes. 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 Probe.

Mixture contains all components except DNA template, primers and probes.

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

	2008-100Q	2008-200Q	storage
1step PCR Mix Probe	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 Probe composition

component
Taq DNA polymerase
MgCl ₂
dNTPs
2x reaction buffer

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

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:

component	PCR reaction volume		
	10 µl	20 µl	25 µl
1step PCR Mix Probe	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*
probe***	0.06 µM**	0.12 µM**	0.15 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 -100 ng RNA	0.1 pg -100 ng RNA
	0.1 pg - 1 ng mRNA	0.1 pg - 1 ng mRNA	0.1 pg - 1 ng mRNA
sterile water	up to 10 µl	up to 20 µl	up to 25 µl

* recommended for standard qPCR
** amount of each probe should be optimized
*** 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 probe/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	15-30 s
	58-70 °C*	15-60 s**

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



A&A BIOTECHNOLOGY

innovating life science

A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 600 776 268, +48 883 323 761
info@aabiotech.com, www.aabiotech.com

