

Medix Biochemica

Product Manual

Cat. No: #6101, #6201Hi, #6201Lo

Volcano3G® RT-PCR Probe 2x Master Mix

Description

Volcano3G® RT-PCR Probe 2x Master Mix contains all components necessary for a successful and reliable real-time RT-qPCR in all standard PCR cyclers, including dNTPs and an optimized reaction buffer.

An aptamer-based hot-start formulation of the Volcano3G® DNA polymerase prevents false amplification. Temperatures above 50°C cause the aptamer's secondary structure to melt and will set-free the polymerase.

Applications include rapid detection and identification of RNA & DNA targets, reverse transcription qPCRs (RT-qPCRs), and qPCRs.

This manual can be used for either Volcano3G® RT-PCR Probe 2x Master Mix (#6101) or the ROX reference dye alternatives Volcano3G® RT-PCR Probe 2x Master Mix (+High ROX) (#6201Hi) and Volcano3G® RT-PCR Probe 2x Master Mix (+Low ROX) (#6201Lo).

Kit components

Component	S pack	M pack
Volcano3G RT-PCR Probe 2x Master Mix	1 x 1.25 mL	5 x 1.25 mL

*Other pack sizes, bulk orders and customization are available upon request.

Storage and shipment

Transport with cool packs. The reagents should be stored at -25°C to -15°C upon arrival. The reagents are stable until the expiration date if stored correctly.

Experimental recommendations for first use:

Run a PCR with a temperature gradient at the RT-step and annealing step in order to find the optimal temperature for your assay.

Most RT-PCR assays work well with a RT-cycling step consisting of a short denaturation followed by incubation at 58-70°C and subsequent PCR cycling.

Reaction Master Mix set-up

The recommended master mix set-up for a 25 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
Volcano3G RT-PCR Probe 2x Master Mix	12.5	1x
Forward primer (10 µM)	1.25	500 nM (50-1000 nM)
Reverse primer (10 µM)	1.25	500 nM (50-1000 nM)
Probe (10 µM)	x	50-1000 nM
Template / Sample extract *	y	>0.1 ng (0.1-2500 ng)
Nuclease-free water	Up to 25 µL final volume	

Keep all components on ice.

Spin down and mix all solutions carefully before use.

This master mix is optimized for an amplicon size between 60-300 bp.

*Recommended template concentration should be 0.004 ng/µl – 0.1 µg/µl (of total RNA or genomic DNA)

Instrument and program set-up

Cycles	Steps	Temperature	Time
RT Cycling			
10	Denaturation	95°C	3 sec
	Reverse Transcription* (temperature to be optimized)	58-70°C	60 sec
PCR Cycling			
35-50	Denaturation	95°C	10 sec
	Annealing / Extension **	58-70°C	50 sec
Hold		<10°C	Hold

*Volcano3G® DNA polymerase allows “zero-step” RT-PCRs directly from RNA templates (without an isothermal reverse transcription step), as reverse transcription also takes place simultaneously with DNA amplification during the cycled PCR elongation step. Thus, a reverse transcription step is optional and can be omitted in some cases.

**A new RT-PCR is ideally established by running a temperature gradient to find the best reverse transcription / annealing / extension temperature for each primer pair. The annealing temperature of a primer is strongly influenced by its nucleic acid sequence and the reaction buffer composition (salts and pH). Since RNA:DNA hybrids are typically more stable than DNA:DNA hybrids, the annealing temperature in the RT-step can be higher than in during PCR cycling.

Volcano3G® DNA polymerase is fully thermostable and most active between 55-95°C.