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 | 1x 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)

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## 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.

## Technical information and support

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.

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