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Volcano3G® RT-PCR GreenDye 2x Master Mix

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Store at -20°C
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Contents

Volcano3G® RT-PCR GreenDye 2x Master Mix contains all components necessary for a successful and reliable real-time RT-qPCR in all standard PCR cyclers, including dNTPs, a fluorescent dsDNA-binding dye and an optimized reaction buffer.
An aptamer-based hot-start formulation of the Volcano3G® DNA polymerase prevents false amplification. Temperatures above 55°C cause the aptamer's secondary structure to melt and will set-free the polymerase.
Without the need for costly fluorescent probes, it is a rapid and cost-effective method to quantify various RNA samples.

Applications

- Rapid detection and identification of RNA & DNA targets
- Reverse transcription qPCRs (RT-qPCRs)
- qPCRs
- Melting curve analysis

Experimental recommendations for first use:

- Run a RT-PCR with a temperature gradient at the annealing / extension step to find the optimal temperature for your assay.

Important notes

- Volcano3G® RT-PCR GreenDye 2x Master Mix works very well also for DNA amplification assays.
- The fluorescence of the dsDNA-binding GreenDye is measured at the usual SYBR/FAM channels.
- A reverse transcription step is only optional. Most RT-PCR assays with Volcano3G® work well with a zero-step RT-PCR protocol without an isothermal reverse transcription step.
- This master mix is optimized for an amplicon size between 60-300 bp.
- Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.

References

Volcano3G® DNA polymerase is based on:
Structure and Function of an RNA-Reading Thermostable DNA Polymerase. Angew. Chem. Int. Ed., 2013; 52: 11935–11939. Blatter, N., Bergen, K., Nolte, O., Welte, W., Diederichs, K., Mayer, J., Wieland, M. and Marx, A.

Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC, 1999/45/EC and 1272/2008 (CLP Regulation) any products which do not contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic, do not require a MSDS. However, we recommend the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLS Biotec takes no liability for damage resulting from handling or contact with this product. This product is not hazardous, not toxic, not IATA-restricted.

Recommendations for RT-PCR/ Reaction Setup

RT-PCR/PCR Mix

Component	Volume	Final concentration
Volcano3G® RT-PCR GreenDye 2x Master Mix *	12.5 µl	1x
Primer forward (10 µM)	1.25 µl	500 nM (50-1000 nM)
Primer reverse (10 µM)	1.25 µl	500 nM (50-1000 nM)
Template/Sample extract **	y µl	>0.1 ng (0.1-2500 ng)
Nuclease-free water	up to 25µl total reaction vol.	

Primer concentrations are suggestions and can be significantly different for your optimized assay.
Keep all components on ice.
Spin down and mix all solutions carefully before use.
* The Volcano3G® RT-PCR 2x Master Mix with GreenDye proved to enable sensitive and reliable detection of RNA without the need for probes.
** Recommended template concentration should be 0.004 ng/µl – 0.1 µg/µl (of total RNA or genomic DNA).

Typical RT-PCR protocol*

Initial denaturation step	95°C	60 sec	
Reverse Transcription (temperature to be optimized)***	58-70°C	600 sec	
Denaturation	95°C	10 sec	35-50 cycles
Annealing/Extension **	various ***	50 sec	
Melting **	60°C – 95°C		
Hold	<10°C	hold	

Suggested incubation temperatures and times are suggestions and can be significantly different for your optimized assay.
* 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.
** The fluorescence of the GreenDye is measured at the usual SYBR/FAM channels.
*** A new RT-PCR is ideally established by running a temperature gradient in order 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).
Volcano3G® DNA polymerase is fully thermostable and most active between 55-95°C.

Product is not from human, animal or plant origin. The source of the product is recombinant protein expression in *E. coli*. The product is for research use only and may be used for *in-vitro* experiments only.

Quality Control Assays

Volcano3G® RT-PCR GreenDye Mix is tested for a successful RT-qPCR performance. A 151 bp fragment (HPRT1 mRNA) is amplified from an RNA dilution series (500.000, 50.000, 5000, 500 copies/rxn) and quantified by the fluorescence of dsDNA-binding GreenDye. The linearity of amplification over the specified serial dilution is demonstrated. Additionally, melting points of the amplification products are analysed. The activity of Volcano3G® DNA polymerase is monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and DNA primer. Enzyme concentration is determined by protein-specific staining. Please inquire more information at info@mypols.de for lot-specific concentration. No contamination has been detected in standard test reactions.

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