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Volcano2G RT-PCR 2x Master Mix

#6100, #6200, #6300, #6400
Store at -20°C

Contents

Volcano2G RT-PCR 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. Hot-start formulation of Volcano DNA polymerase prevents false amplification during the reaction set-up.
#6100 **Volcano2G RT-PCR Probe 2x Master Mix** is suitable for all probe-based qPCR assays.
#6200 **Volcano2G RT-PCR Probe 2x Master Mix (+ROX)** is suitable for all probe-based qPCR assays requiring a passive reference dye.
#6300 **Volcano2G RT-PCR GreenDye 2x Master Mix** is suitable for all real-time dye-based qPCR assays.
#6400 **Volcano2G RT-PCR GreenDye 2x Master Mix (+ROX)** is suitable for all real-time dye-based qPCR assays requiring a passive reference dye.

Applications

- Rapid detection and identification of RNA targets
- Reverse transcription PCRs (RT-PCRs)
- Real-time RT-qPCRs
- qPCRs

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
Volcano2G RT-PCR 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*	x µl	>1 ng (1-1000 ng)
Nuclease-free water	up to 25µl total reaction vol.	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Suggested template concentration should be 0.1 ng/µl - 1 µg/µl (total RNA).

Typical 0-step RT-PCR protocol (an isothermal reverse transcription step is not needed)

Initial denaturation	95°C	2 min	
Denaturation	95°C	15 sec	
Annealing/Extension*	various	45 sec	(25-40 cycles)
Hold	<10°C	hold	

A two-step as well as three-step PCR protocols can be used.
*A new RT-PCR is ideally established by running a temperature gradient in order to find the best 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).
Volcano2G DNA polymerase is most active between 50-95°C

References

Volcano2G 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.

Quality Control Assays

RT-PCR activity: Volcano2G RT-PCR Mix is tested for a successful RT-qPCR performance. A 151 bp fragment (HPRT1 mRNA) is amplified from human total RNA extract and linearity between different template dilutions confirmed.
DNA polymerase activity: Volcano2G polymerase activity 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 the lot-specific concentration.
No contamination has been detected in standard test reactions.

Licences/Patents/Disclaimers

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The product is for research use only and may be used for in-vitro experiments only.

Product source: recombinant protein expression in E.coli.

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Important notes

- Volcano2G RT-PCR 2x Master Mix is optimized for an amplicon size between 60- 300 bp.
- Volcano2G RT-PCR 2x Master Mix is available for all real-time probe-based assays as well as GreenDye-based assays, both with and without ROX as a passive reference dye.
- 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.

Safety

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally 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. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL