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Volcano2G DNA polymerase

#8100
Store at -20°C

Contents

Volcano2G DNA polymerase is supplied as a 5 U/µl solution containing glycerol. It comes together with an 5x optimized reaction buffer. An aptamer-based hot-start formulation of the Volcano2G DNA polymerase prevents false amplification. Temperatures above 50°-55°C cause the aptamer's secondary structure to melt and will set-free the polymerase.

Description

Volcano2G DNA polymerase is an engineered, extremely thermostable reverse transcriptase and combined DNA polymerase, obtained through directed, artificial evolution. Volcano has a half-life at 95°C of >40 min.
The Volcano2G reverse transcriptase and DNA polymerase facilitates "zero-step" RT-PCRs directly from RNA templates (without an isothermal reverse transcription step), as reverse transcription takes place simultaneously with DNA amplification during the cycled PCR elongation step. This also allows reverse transcription reactions at high temperatures, thus minimizing the problems encountered with strong secondary structures in RNA that melt at elevated temperatures.

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
Primer forward (10 µM)	1 µl	0.5 µM (50-1000 nM)
Primer reverse (10 µM)	1 µl	0.5 µM (50-1000 nM)
dNTPs (10mM)	2 µl	400 µM
5x Volcano buffer	10 µl	1x
Volcano2G DNA polymerase (5 U/µl)	1 µl	5 Units/reaction
Template/Sample extract*	various	>1 ng (1-1000 ng)
Nuclease-free water	up to 50 µl total reaction volume	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
*Recommended final template concentration is between 5-500 ng/µl.

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)
Optional melting step		

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 influenced by its nucleic acid sequence and the reaction buffer composition (salts and pH).
Volcano2G DNA polymerase is most active between 50-95°C.

Related Products

Volcano2G is also available as a ready-to-use, real-time RT-qPCR 2x reaction mix: Volcano2G RT-PCR 2x Master Mix, article numbers #6100-6400.

Quality Control

RT-PCR activity: Volcano2G is tested for a successful RT-PCR performance. A 151 bp fragment (HPRT1 mRNA) is amplified from human total RNA extract in a PCR cycler and visualized as a single amplified product.
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.

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.

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Important notes

- Volcano2G DNA polymerase is engineered and optimized for an amplicon size between 60- 300 bp.
- Volcano2G DNA polymerase can also be used for real-time cycling, when adding a suitable real-time PCR dye or a fluorescent probe.
- Volcano2G DNA polymerase does not require Mn^{2+} for optimal reverse transcription activity. However, some assays may be further optimized by the addition of Mn^{2+} to the reaction (suggested concentration is 0.5 - 1 mM).

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.

Licences/Patents/Disclaimers

Product source: recombinant protein expression in E.coli.