www.mypols.de
support@mypols.de
T +49(0)7531 122 965 00

VolcanoCell2G RT-PCR 2x Master Mix

including separate lysis buffer

#7101

Storage

Please store the product upon arrival at -20°C. 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.

	Product size	
Component	**100 reactions**	**500 reactions**
VolcanoCell2G RT-PCR 2x Master Mix	1 x 1.25 ml	5 x 1.25 ml
Lysis buffer (50x)	1 x 1.25 ml	3 x 1.25 ml

Description

This mix has been specifically optimized for direct-from-cell applications.
Skip the time-consuming and expensive RNA purification. The VolcanoCell2G RT-PCR Mix enables you to quantify and detect RNA directly from a cell suspension, or a cell lysate, without a need for purification steps. Simply detach the cells, add the cell suspension directly to your RT-PCR reaction mix, place in your real-time instrument, and start. Optionally, use our lysis buffer to lyse the cells directly in the well or flask, spin down and add the supernatant directly to your RT-PCR mix. The lysates can also be frozen and stored at -20°C.

The included Volcano2G DNA polymerase was engineered through multiple generations of directed, artificial evolution and has a half-life at 95°C of >40min. It facilitates a "zero-step" RT-PCR directly from cell suspension or lysate without an isothermal reverse transcription step.
VolcanoCell2G RT-PCR 2x Master Mix contains all components necessary for a successful and reliable RT-PCR in all standard cycler instruments. It can be used for real-time PCR, when adding a suitable fluorescent probe.
Recommended amplicon size should be between 60-300 bp.
Please note that primers which span an exon-exon junction must be used in order to limit the amplification only to mRNA targets.

Quality Control Assays

VolcanoCell2G RT-PCR 2x Master Mix is tested for a successful RT-PCR performance. A 151 bp fragment (HPRT1 mRNA) is amplified from human total RNA extract and visualized as a single amplified product. The activity of Volcano2G 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 the lot-specific concentration.

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. 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.

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

PCR setup and protocol

Please note that primers which span an exon-exon junction must be used in order to limit the amplification only to mRNA targets.
We recommend using an RNA extract as a positive control.
RNA-specific primers are binding on exon-exon junctions. For instance, you can simply use one of the free primer design tools in the internet, such as primer-blast on the homepage: www.ncbi.nlm.nih.gov/tools/primer-blast/. Ensure that you select the option: "primers must span an exon-exon junction". Primers designed with this parameter will limit amplification to mRNA only.

VolcanoCell2G RT-PCR 2x Master Mix can be used for real-time PCR, when adding a suitable fluorescent probe.

PCR Mix

Component	Volume	Final concentration
VolcanoCell2G RT-PCR 2x Master Mix	12.5 µl	1x
Primer forward (10 µM) *	1 µl	0.4 µM (0.05- 1 µM)
Primer reverse (10 µM) *	1 µl	0.4 µM (0.05- 1 µM)
Probe (10 µM)	1 µl	0.4 µM (0.05- 1 µM)
Template**:		
Cell suspension (in nuclease-free water)	1 – 9.5 µl	<10,000 cells/reaction
or		
cell lysate (in 1x lysis buffer)	2.5 µl	<10,000 cells/reaction
Nuclease-free water	up to 25µl total reaction volume	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Please note that primers which span an exon-exon junction must be used in order to limit the amplification only to mRNA targets.
** Suggested cell number is 10 – 10,000 cells, lysed or prepared in nuclease-free water, and further diluted in nuclease-free water. Cell suspensions should be prepared fresh as suggested in the METHODS section of this manual.

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

Initial denaturation	95°C	3 min	
Denaturation	95°C	15 sec	
Annealing/Extension*	55–75°C	60 sec	(25-40 cycles)
Hold	<10°C	hold	

* Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used. A new PCR is ideally established by running a temperature gradient in order to find the best annealing/extension temperature for each primer pair. Also a three-step protocol can be applied with separate annealing and extension steps.

METHODS

VolcanoCell2G has been optimized using 10 – 10,000 cells per reaction. It is compatible with a wide range of mammalian cell lines and primary cells.
For best results we recommend first determining the appropriate cell number for your particular experiments by testing a cell range between 10-10,000 cells (e.g.: 100; 1,000; 2,500; and 5,000 cells/reaction).
We recommend using a maximum of 10,000 cells per reaction as higher numbers may lead to low fluorescence signals or inhibition.
Use appropriate sterile technique when handling cells and avoid any possible contamination with foreign RNA/DNA.

Template from cell cultures can be prepared either directly by diluting cell pellet in nuclease-free water (Protocol 1) or by lysing cells in the well or tube in 1x VolcanoCell2G lysis buffer (Protocol 2) which may be more useful for high-throughput assays when RT-PCR reactions need to be prepared from separate wells (eg.: on a microtiter plate).

Protocol 1
Cell suspension preparation with nuclease-free water (cell detachment necessary)

For adherent cell cultures:

1. Carefully remove all media from cell culture dish/flask and add an appropriate volume of 1x PBS (use 10 ml PBS for 10 cm dish or T75 flask) to wash the cells.
2. Remove the PBS and add trypsin to detach your cells (use enough trypsin to cover the cells e.g. 1 ml trypsin for a 10 cm dish or 3 ml trypsin for a T75 flask).
3. Incubate at 37°C or at room temperature and check cell detachment under a microscope every 2 min until you see the cells have all detached.
4. Once the cells have detached, add medium containing serum to inactivate trypsin (for each 1 ml of trypsin add 2 ml of serum-containing medium).
5. Transfer the cell suspension to a centrifuge tube and centrifuge at 1,300 rpm for 3 min at room temperature to pellet the cells.
6. Remove the supernatant and resuspend the cell pellet in 1 ml - 10 ml 1x PBS according to the size of the pellet to determine the total cell count (this will also wash the cells and get rid of any remaining medium).
7. Count the cells from ~10 µl of the cell suspension using a hemocytometer counting chamber or an electronic counter.
8. Centrifuge again at 1,300 rpm for 5 min to pellet the cells.
9. Now prepare the template for your PCR reaction: Aspirate the supernatant and resuspend the pellet in an appropriate amount of nuclease-free water to achieve cell density of max. 5,000 cells/µl (for a final cell number of 10,000 cells/reaction). Alternatively, prepare several tubes of different cell

densities ranging from 50 to 5,000 cells/µl (100 – 10,000 cells/reaction) in order to investigate the optimal number of cells per reaction for your future experiments. Keep the suspensions on ice and always prepare fresh before RT-PCR.

10. Use this as template in the RT-PCR.

For suspension cell cultures: skip steps 1-4 and start with step 5 above.

Protocol 2
Cell suspension preparation with VolcanoCell2G lysis buffer (no cell detachment necessary)
Note: Dilute 50x VolcanoCell2G lysis buffer with nuclease-free water to a final concentration of 1x.

For adherent cell cultures:

1. Carefully remove all media from the cells by pipetting off the supernatant as thorough as possible.
2. Place the cell culture plate on ice and keep on ice during further steps.
3. Add the **1x VolcanoCell2G lysis buffer** (depending on cell number*) directly to the plate.
4. Incubate for 15 minutes on ice. Slightly shake the plate occasionally to dispense the lysis buffer. Depending on the cell type, some (or all) cells may remain adherent to the culture dish – do not scrape or remove them but use only their supernatant for further steps.
5. Transfer the supernatant from well or plate into a tube and centrifuge at approx. 10,000 rpm for 30 sec at room temperature to pellet any remaining cell debris.
6. Use the supernatant to prepare several dilution series in order to investigate the optimal lysate concentration per reaction for your future experiments.
7. Use this as template in the RT-PCR. Alternatively the supernatant can be frozen and stored at -20°C or -80°C. Please avoid multiple freeze-thaw cycles (<5 times) of the lysate.

For suspension cell cultures:

1. Pellet cells by centrifugation and remove the supernatant.
2. Transfer the pellet on ice and keep on ice during further steps.
3. Add the **1x VolcanoCell2G lysis buffer** (depending on cell number*) and resuspend the cell pellet by gently pipetting up and down.
4. Incubate the mix for 15 minutes on ice. Slightly shake the tube occasionally.
5. Centrifuge the cells at approx. 10,000 rpm for 30 sec at room temperature to pellet the cells and follow step 6 and step 7 above.

*We recommend 1 ml VolcanoCell2G Lysis Buffer for about 1 million cells. This corresponds to approximately 2.500 cells per reaction.