

DirectBlood Genotyping PCR Kit

#5000

Storage:

The freeze-dried DirectBlood Genotyping PCR LyoCake can be stored at room-temperature or at -20°C once it has been rehydrated. Please store the included rehydration buffer upon arrival at -20°C.

Contents and Description:

The kit contains all components necessary for rapid, sensitive and reproducible detection of SNPs from EDTA blood samples without previous DNA isolation.

The lyophilized 2x DirectBlood Genotyping PCR Mix includes an engineered, hotstart formulated DNA polymerase, an optimized buffer and ultrapure dNTPs.

Quality Control Assays

DirectBlood Genotyping PCR Kit is tested for functional and accurate genotyping PCRs direct from EDTA blood samples.

DNA polymerase activity: DNA polymerase activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.

Enzyme-concentration has been 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.

Preparations before use:

Please rehydrate the lyocake by adding exactly **218µl** of the respective **Rehydration Buffer** to the PCR mix, resulting in 250µl of ready-to-use 2x Master Mix. Subsequently invert the closed tube a few times or briefly vortex and spin down the mixture before use. Tube should be placed on ice after rehydration. The rehydrated 2x DirectBlood Genotyping PCR Mix is now ready to be used for setting up a PCR experiment or can be stored at -20°C.

Sample preparation:

Dilute a given EDTA blood sample to 2% (v/v) in DNase-free water by adding of 10 µL blood to 490 µL water. Subsequently invert the closed tube a few times or briefly vortex the mixture before use. Samples can also be stored for a couple of weeks at 4°C, e.g., for re-testing purposes.

PCR Reaction Setup

Component	Volume	Final concentration
2x DirectBlood Genotyping PCR Mix	10 µL	1x
4x Primer/Probe Mix	5 µL	1x
Diluted blood sample (2%)	5 µL	0.5%
Total volume/reaction	20 µL	

Suggested PCR protocol

Initial denaturation	95°C	2 min	
Denaturation	95°C	10 sec	
Annealing/Extension*	60°C	40 sec	(50 cycles)

* Fluorogenic Data should be collected during this step. Real-time PCR signals may be reduced to 25% of the signals obtained from extracted DNA samples. This is a normal result and will still be leading to significant, valid Ct-values and unambiguous melting peaks.

Please note:

Suggested cycling protocols can vary and depend strongly on the cyclers and applied PCR assay.

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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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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Real-time PCR detection setup:

The DirectBlood Genotyping Kit is compatible with a wide range of assays. It has been successfully tested with a variety of hydrolysis probes and hybridisation probes.

Cycler compatibility

This product is compatible for the use with any qPCR cycler not requiring a passive reference dye (not included in the kit).

Recommendations for sample handling

- Spin down and mix all solutions carefully before use.
- Include a control without sample as a negative control.
- Minimize the number of freeze-thaw cycles by storing the rehydrated product in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.
- We recommend the use of disposable gloves, DNase and RNase free filter tips and plastics.

This product is also available with already established SNP-assays. Please request #5100.