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# DirectBlood Genotyping PCR Kit

#5000

## Storage

- Please store the included Rehydration Buffer upon arrival at -20°C.
- The freeze-dried DirectBlood Genotyping PCR LyoCake is stored at room-temperature. Once rehydrated, please store the PCR mix at -20°C.

## Contents and Description

The kit contains all components necessary for rapid, sensitive and reproducible real-time PCR detection of SNPs from EDTA blood samples without previous DNA isolation. Only target-specific primers and probes have to be added.
The lyophilized 2x DirectBlood Genotyping PCR Mix includes an engineered, hotstart formulated DNA polymerase, optimized buffer components and ultrapure dNTPs. Additionally, the kit consists of a Rehydration Buffer.

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## Quality Control

All components of the DirectBlood Genotyping PCR Kit are tested for functional and accurate genotyping PCR results. For more information, please inquire at info@mypols.de.

## 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.

## Licences/Patents/Disclaimers

This product is for the purchaser's own internal research use and may not be resold, modified or used for production and commercial purposes of any kind without an agreement with myPOLS Biotec. For information on obtaining additional rights, please contact: info@mypols.de.
The product is for research use only and may be used for in-vitro experiments only.

Product source: recombinant protein expression in *E.coli*

## I. – Preparations before use

1. Rehydrate the lyocake by adding exactly **218 µl** of the included **Rehydration Buffer** onto the lyocake, resulting in 250 µl of ready-to-use 2x Master Mix.
2. Subsequently invert the closed tube a few times, briefly vortex and spin down the mixture before use. The tube should be placed on ice after rehydration.
3. The rehydrated 2x DirectBlood Genotyping PCR Mix is then ready to be used or stored at -20°C.
4. Program PCR cycler as described in II., please see below

## II. – Recommended real-time PCR protocol

Program the PCR cycler with the following protocol:

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

Please note:
PCR protocol and detection channels may be adjusted according to the applied primers and probes mix.
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).

## III. – Recommended sample preparation

1. Dilute a given EDTA blood sample 2% (v/v) in DNase-free water by adding 10 µL of blood sample to 490 µL water.
2. 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.

**Optional step:**

3. Heat the diluted EDTA blood sample to 80°C for ≥5 minutes to reduce potential infectivity of the blood sample.
4. Spin down the sample for 1-2 seconds but do not centrifuge after this step anymore!

## IV. – Recommended PCR Reaction setup

1. Prepare the PCR reaction as follows:

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

Please note, we recommend to include one positive control and one negative control reaction in each PCR run.

2. Insert PCR-plate or strips with prepared PCRs and start run

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