

Medix Biochemica

Product Manual
Cat. No: #5000

DirectBlood Genotyping PCR Kit

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, hot-start formulated DNA polymerase, optimized buffer components and ultrapure dNTPs. Additionally, the kit consists of a Rehydration Buffer for rehydration of the LyoCake.

Kit components

Component	S pack	M pack
DirectBlood Genotyping PCR Kit	1 x bag (containing 4 x LyoCakes)	5 x bags (each containing 4 x LyoCakes)
Rehydration Buffer	1 x 1.25 mL	5 x 1.25 mL

*Other pack sizes, bulk orders and customization are available upon request.

Storage and shipment

Transport with cool packs. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly. Protect all components from light.

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.

Recommended sample preparation

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

Reaction Master Mix set-up

The recommended master mix set-up for a 20 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
2x DirectBlood Genotyping PCR Mix	10	1x
4x Primer/Probe Mix	5	1x
Diluted blood sample (2%) or control sample	5	0.5%

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

Instrument and program set-up

Cycles	Steps	Temperature	Time
1	Initial denaturation	95°C	2 min
50	Denaturation	95°C	10 sec
	Annealing / Extension	60°C	40 sec