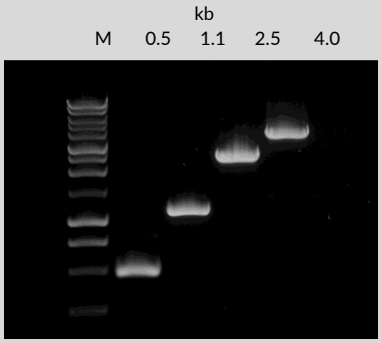
EURx® MOLECULAR BIOLOGY PRODUCTS

Direct Blood PCR Kit

Cat. No.	size
E0950-01	100 reactions of 20 µl
E0950-02	500 reactions of 20 µl

Storage Conditions: Store at -20°C.



PCR amplification using EURx Direct Blood PCR Kit.

Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder.

Lanes 0.5 to 4 kb: PCR amplification reactions directly from whole human blood (5% in the reaction), using human-specific primers and EURx Direct Blood PCR Kit.

Direct Blood PCR Kit enables to perform PCR reactions directly from whole blood with no prior DNA extraction or purification.

Description:

- The Direct Blood PCR Kit allows to use whole blood stored at +4°C or -20°C as well as dried blood spots on cards such as commercially available Whatman FTA® cards.
- Blood can be preserved with all most often used anticoagulants: EDTA, citrate or heparin.
- It is possible to use whole blood in a wide range of concentrations: from 1% to 20%. The recommended starting point is 5% ($MgCl_2$ concentration contained in the Blood PCR buffer is optimized for 5% blood).
- The Direct Blood PCR Kit employs a genetically engineered thermophilic DNA polymerase with high tolerance to blood inhibitors.
- Blood DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in 50-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to use shorter extension times.
- **Due to the genetic modification of the polymerase, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols**.
- The Direct Blood PCR Kit allows to obtain a wide range of product size (over 4 kb).

Direct Blood PCR Kit contains:

1. 2 x Blood PCR Master Mix
2. Blood DNA Polymerase
3. 25 mM $MgCl_2$
4. 50 mM EDTA pH 8.0
5. DMSO
6. Water, nuclease free

2 x Blood PCR Master Mix:

The master mix contains 2 x concentrated optimized PCR buffer, 6 mM $MgCl_2$ and dNTPs.

Blood DNA Polymerase Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.5% Tween™20, 0.5% Igepal CA-630, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

Quality Control:

All preparations are assayed for contaminating 3'-exonuclease, and nonspecific single - and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

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Preparation of PCR Reaction From Whole Blood:

PCR premix:

Component	20 µl reaction	50 µl reaction	Final concentration
2 x Blood PCR Master MIx - contains 6 mM $MgCl_2$	10 µl	25 µl	1x 3.0 mM $MgCl_2$
Primer A	Variable	Variable	0.5 µM
Primer B	Variable	Variable	0.5 µM
Blood DNA Polymerase	0.4 µl	1 µl	
Sterile double-distilled water	Add to 19 µl	Add to 47.5 µl	
Premix volume	19 µl	47.5 µl	
Mix a PCR premix thoroughly. Centrifuge briefly. Add blood to the bottom of the tube. Blood is heavier than the PCR premix and settles to the bottom. Do not mix blood with the premix!			
Whole blood	1 µl	2.5 µl	5%
Total volume	20 µl	50 µl	

Preparation of PCR Reaction From Blood Spot Cards:

Put a 1-2 mm punch in a PCR tube. Wash the punch by adding 100 µl of sterile H_2O to the tube and pipetting up and down several times. Completely remove and discard H_2O.

Prepare a PCR premix in a separate tube.

PCR premix:

Component	20 µl reaction	50 µl reaction	Final concentration
2 x Blood PCR Master MIx - contains 6 mM $MgCl_2$	10 µl	25 µl	1x 3.0 mM $MgCl_2$
Primer A	Variable	Variable	0.5 µM
Primer B	Variable	Variable	0.5 µM
Blood DNA Polymerase	0.4 µl	1 µl	
Sterile double-distilled water	Add to 20 µl	Add to 50 µl	
Premix volume	20 µl	50 µl	
Mix all components of the PCR premix thoroughly. Centrifuge briefly. Add the PCR premix to the washed punch. Do not mix. Centrifuge briefly.			
1-2 mm punch of a blood spot card			
Total volume	20 µl	50 µl	

Optional components of PCR reaction:

Component	20 µl reaction	50 µl reaction	Final concentration
25 mM $MgCl_2$	0.4-1.2 µl	1-3 µl	3.5-4.5 mM
50 mM EDTA	0.4-0.8 µl	1-2 µl	1-2 mM
DMSO	0.4-0.8 µl	1-2 µl	2-4%

Notes:

1. Blood can be stored at +4°C for up to 3 months. For long term storage, it is recommended to store blood at -20°C or dried on blood spot cards (such as Whatman FTA® cards). Blood can be preserved with EDTA, citrate or heparin.
2. It is possible to use a wide range of blood concentrations (from 1% to 20%) in the reactions with the Direct Blood PCR Kit. The recommended starting point is 5% ($MgCl_2$ concentration contained in the Blood PCR buffer is optimized for 5% blood).
3. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration.
4. PCR reactions should be set up on ice.
5. PCR reaction from whole blood:
   Prepare a PCR premix first (all components except blood). Mix the PCR premix well. Centrifuge briefly to settle down the reaction components and remove bubbles. Add whole blood to the bottom of a tube. Do not mix blood with the PCR premix.
6. PCR reaction from blood spot cards:
   Put a 1-2 mm punch in a PCR tube. Wash the punch by adding 100 µl of sterile H_2O to the tube and pipetting up and down several times (4-6 times). Completely remove and discard H_2O.
   Prepare a PCR premix in a separate tube (all components except the blood spot punch). Mix all components of the PCR premix thoroughly. Centrifuge briefly to settle down the reaction components and remove bubbles. Add the PCR premix to the washed punch. Do not mix. Centrifuge briefly.
7. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
8. The 2 x Blood PCR Master Mix provides 3 mM $MgCl_2$ in the final reaction. 3 mM $MgCl_2$ is optimal for most targets using 5% blood and for some targets using 10% blood. $MgCl_2$ optimization may be required especially when higher blood percentage (≥10%) is used. If increased Mg^{2+} concentration is needed use 25 mM $MgCl_2$ provided to adjust the concentration up to 4.5 mM.
9. Excess of $MgCl_2$ may cause unspecific products are created. In such cases add from 1 to 2 µl of 50 mM EDTA (included in the kit) per 50 µl reaction to decrease effective $MgCl_2$ concentration. It is most often necessary when blood is used in lower concentration than 5% (1-4%).
10. 1 µl of Blood DNA Polymerase is recommended amount of the enzyme per 50 µl amplification reaction and works well with most amplicons. For some PCR targets further optimization will be required. If unspecific bands are created try to decrease amount of the enzyme to 0.75 µl per 50 µl reaction volume.
11. In most cases there is no need to add additives to PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO (provided) can be included to improve amplification. The recommended starting DMSO concentration (if needed) is 2%.
12. **After PCR, centrifuge the reactions at maximum speed for 1 min to pellet debris from blood.**

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Thermal Cycling Conditions:

Step	2-step protocol		3-step protocol		Number of Cycles
	Temp.	Time	Temp.	Time	
Lysis of Cells, Initial	98°C	5 min	98°C	5 min	1
Denaturation	98°C	5-10 s	98°C	5-10 s	35-40
Annealing	-	-	X°C	10-30 s	
Extension	72°C	30 s/1 kb	72°C	30 s/1 kb	
Final	72°C	1 min	72°C	1 min	1
Cooling	4°C	Indefinite	4°C	Indefinite	1

Notes:

1. A 5-minute initial denaturation at 98°C enables lysis of leukocytes.
2. Blood DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (T_m) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. T_m's should be calculated with the base-stacking method (nearest-neighbor method) using calculator on the website: http://eurx.com.pl/CALC/. Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule use an annealing temperature at a T_m +2°C of the lower T_m primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. A 2-step protocol allows to perform combined annealing/extension step at 72°C and is recommended for primers with T_m values of at least 70°C. The 2-step protocol allows to save time of PCR reaction.
4. Extension time of 30 s/ 1 kb is recommended for most targets.

Troubleshooting:

Nonspecific products

- Increase annealing temperature.
- Add 50 mM EDTA (1-2 µl/50 µl reaction).
- Decrease amount of Blood DNA Polymerase in the reaction.
- Shorten extension time.
- Increase the percentage of blood in the reaction.
- Design new primers.

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