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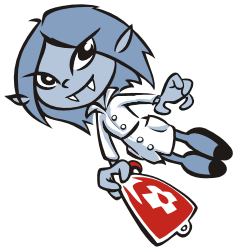


Direct Blood PCR Kit

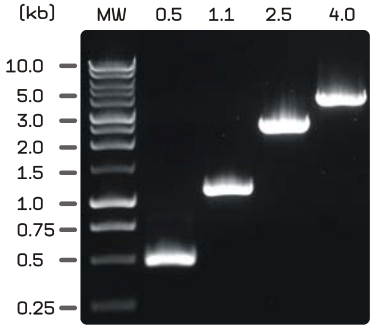
Direct Blood PCR Kit

Cat. No. **Size**

E0950-01 100 reactions 20 µl each

E0950-02 500 reactions 20 µl each

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 (E3131). 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 contains:
1. [2x] Blood PCR Master Mix
2. Blood DNA Polymerase
3. $MgCl_2$ [25 mM]
4. EDTA pH 8.0 [50 mM]
5. DMSO
6. Water, nuclease free

Direct Blood PCR Kit enables to perform PCR reactions directly from whole blood without 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 thermostable 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 3'→5' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- 5'→3' exonuclease activity is not present. When encountering a stretch of double-stranded DNA upon polymerization, the enzyme halts.
- 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).

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.

[2x] Blood PCR Master Mix:

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

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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Direct Blood PCR Kit

PCR PROTOCOL (1)

Preparation of PCR Reaction from whole blood:

Component	Volume / 20 µl Reaction	Volume / 50 µl Reaction	Final concentration
2x Blood PCR Master Mix, containing 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	1.0 µl	
H_2O	Add to 19 µl	Add to 47.5 µl	
Premix Volume	**19 µl**	**47.5 µl**	
Mix 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 % [v/v]
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 H2O to the tube and pipetting up and down several times. Completely remove and discard H2O. Prepare a PCR premix in a separate tube.			
Component	**Volume / 20 µl Reaction**	**Volume / 50 µl Reaction**	**Final concentration**
2x Blood PCR Master Mix, containing 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	1.0 µl	
H_2O	Add to 20 µl	Add to 50 µl	
Premix Volume	**20 µl**	**50 µl**	
Mix 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	Volume / 20 µl Reaction	Volume / 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	1.0-2.0 mM
DMSO	0.4-0.8 µl	1-2 µl	2-4 %[v/v]

Notes:

1. **Storage:** 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. **Blood Concentration:** 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. **Thaw and Mix:** Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration.
4. **On Ice:** PCR reactions should be set up on ice.
5. **PCR reaction from whole blood:**

   Prepare a PCR premix first (all components except blood). Blood DNA Polymerase should be the last component added to the PCR premix. In the absence of dNTPs, proofreading activity of Blood DNA Polymerase may degrade primers. 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). Blood DNA Polymerase should be the last component added to the PCR premix. In the absence of dNTPs, proofreading activity of Blood DNA Polymerase may degrade primers. 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. **Preheated Cycler:** Place reactions in a thermal cycler that has been preheated to denaturation temperature.
8. **$MgCl_2$:** The Blood PCR Buffer 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. Adding 1 µl of a 25 mM $MgCl_2$ solution will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 1.25 mM (20 µl) or 0.5 mM (50 µl).
9. **EDTA:** 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. **Polymerase Concentration:** 1 µl of Blood DNA Polymerase is the 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. **PCR Optimization:** 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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PCR PROTOCOL (2)

Thermal Cycling Conditions:

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

Notes:

1. **Cell Lysis:** A 5-minute initial denaturation at 98˚C enables lysis of leukocytes.
2. **Annealing:** Blood DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (Tm) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. Tm's should be calculated with the base-stacking method (nearest-neighbor method). Use the calculator of the base-stacking method on Roboklons website (www.roboklon.de/eurx/blood-pcr). Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule, for primers →20nt, use an annealing temperature at a Tm +3˚C of the lower Tm primer. For primers 20nt, use an annealing temperature equal to the Tm of the lower Tm primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. **Two-Step Protocol:** A 2-step protocol allows to perform combined annealing/extension step at 72˚C and is recommended for primers with Tm values of at least 69˚C (→20nt) or 72˚C (‹20nt). The 2 step protocol allows to save time of PCR reaction.
4. **Extension:** 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.