



**The ENZYME Company**

## InBlood Polymerase

**Cat.No.: 108510      1000 units**

InBlood DNA polymerase is a mixture of several proprietary thermostable polymerases designed for effective PCR of templates directly from blood, without DNA purification. Polymerases are artificially created in the laboratory by genetic engineering techniques with the use of modern protein design approaches.

Using InBlood DNA polymerase PCR fragments may be obtained directly from the reaction mixture containing up to 25% of human blood without DNA isolation reducing contamination risk, labor time and the total cost of the procedure.

InBlood DNA polymerase can effectively produce PCR fragment up to 1.5 kb in length at 1-25% (v/v) of blood in the reaction mixture. Hot Start feature provides high sensitivity of PCR tests with increased specificity and low background level.

InBlood polymerase was proved to direct effective PCR from human blood, from animal blood as mouse, rat, cat, dog, cow, sheep, pig and from blood of some birds as hen and pigeon.

**Storage Conditions:    Long term storage: -20°C**

**Concentration:            20-25 units/μl**

### Features

- 5'-3' polymerase activity
- 3'-5' exonuclease (Proof-reading) activity
- High specificity
- Hot start
- Resistance to inhibitors from blood

### Limitations

- InBlood polymerase is not recommended for PCR of fragment longer than 1,5 kb
- InBlood polymerase can't be used for blood if the blood contains heparin or ACD
- InBlood polymerase can be used for the detection of SNP, meanwhile ask Bioron for better enzyme for SNP detection – SNPase or InBlood SNPase (available soon).

### Reaction components

#### Blood

Fresh or frozen blood collected with/without EDTA or Na-citrate can be used for PCR. Blood spots on paper/swabs can be used as well.

- Blood should be collected into tubes with EDTA or Na-citrate according to the standard protocols. Blood can be stored at +4°C up to 15 days;
- Fresh or frozen blood can be used for the analysis. Fresh blood should be used before clot formation. Frozen blood should be defrosted and mixed well before analysis.
- Optimal concentration of human blood in the reaction mixture is 5-10%. Maximal concentration of human blood in the reaction mixture is 20-25%. If concentration is higher than 15% the solution starts to form precipitate and pipetting of the solution may be complicated. The best results can be obtained if EDTA is used as anticoagulant.
- Optimal concentration of animal blood should be found in experiment for each particular species.

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- If blood spot on the paper is used, the piece of paper (approx. 1mm) should be plunged completely into the reaction solution.

#### **MgCl<sub>2</sub> concentration**

- Enzyme is provided together with the buffer which provide 3.5 mM of MgCl<sub>2</sub> and this is optimal for the majority of reaction
- If more than 10% of Na-citrate is added to the blood sample, the final concentration of MgCl<sub>2</sub> should be increased 1-2 mM (up to 4.5-5.5mM)
- If the highest concentration of blood is used (20-25%), the final concentration of MgCl<sub>2</sub> should be increased 1-2 mM (up to 4.5-5.5mM)

#### **Reaction volume**

The reaction volume should not be less than 25 microliters in any case. We recommend to use reaction volume 50 microliters if concentration of blood in the reaction mixture is 20-25%.

#### **PCR protocol for blood**

1. Defreeze the blood and mix thoroughly
2. Mix the reaction component as described in Table 1

Table 1

Component	25 µl	50 µl	Final concentration
5x buffer	5 µl	10 µl	1x
10mM dNTP (each)	0,5 µl	1 µl	0,2 mM
Primer 1	-	-	0,3 µM
Primer 2	-	-	0,3 µM
InBlood Polymerase	0,5 µl	1 µl	1x
Blood	1-5 µl	2-10 µl	4-20%
Sterile Water	Up to 25 µl	Up to 50 µl	-

#### **Attention!**

Add blood at the last step of reaction mixture preparation. Before blood addition, mix all components of the reaction mixture by pipetting, centrifuge briefly. Add aliquot of the blood to the reaction mixture by single pipetting-out. Do not mix the blood with the reaction mixture! Let the blood to be loaded on the bottom of the tube. If FDA filters with blood spots are used, incubate the filters 5 min at 50 °C in water, remove water and place the filters into the reaction mixture.

3. Put the tubes carefully (without shaking) into the PCR cycler. Run the program as shown below:
  - A. Pre- denaturation - 2-3 min 95 °C
  - B. Denaturation – 30 sec 95 °C
  - C. Annealing-Elongation 30-45 cycles

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Annealing – 20-30 sec (T<sub>m</sub>) – annealing temperature of the primers

Elongation – 1-2 min 68 °C

D. Final elongation – 1 min 68 °C

*Remarks*

- Pre-denaturation is required for the release of DNA from cells.
  - Purified DNA requires 5-7 cycles less for the same final amount of PCR product in comparison with blood.
4. If 25 microliters reaction volume is used, centrifuge the sample after the cycling 5 min at max. speed of microcentrifuge, the debris should form pellet on the bottom of the tube. Use the supernatant for electrophoresis in agarose gel.

<b>Catalog #</b>	<b>conc.</b>	<b>Pack size</b>	
108510	20-25 u/μl	1000 u	
108550	20-25 u/μl	5000 u	

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