Ampliqon

PCRonBlood™ for Human Blood

For 100 Reactions of 50µl pr Reactions

Cat. No.: 400200 (100 Reactions)

Cat. No.	Product Name	Size Reac	5x PCR on Blood-Hu	5x PCR on Blood Enhancer-Hu	Taq DNA Pol	dNTP (5 mM)
400100	PCRonBlood™ Human Blood	100	1 mL	1 mL	50 µl	200 µl
400200	PCRonBlood™ Human Blood	100	1 mL	1 mL	not included	not included

Cat. No.	Product Name	Size Reac	5x PCR on Blood-Mo	5x PCR on Blood Enhancer-Mo	Taq DNA Pol	dNTP (5 mM)
410100	PCRonBlood™ Mouse Blood	100	1 mL	1 mL	50 µl	200 µl
410200	PCRonBlood™ Mouse Blood	100	1 mL	1 mL	not included	not included

Store at -20°C. *Reagent for in-vitro laboratory use only*

General Description

Mammalian body fluids including blood contain many substances that inhibit the activity of enzymes such as *Taq* DNA Polymerase. Therefore it is generally necessary to remove the inhibitory substances and purify DNA before performing DNA analysis on cells present in these samples. Traditional preparation of DNA samples from mammalian cells includes overnight treatment with proteinase K, followed by purification by phenol/chloroform extraction and EtOH precipitation. Recently, many kinds of DNA isolation kits have become commercially available, so DNA isolation has become simpler than before, but is still time-consuming. Finally, the DNA isolation procedure generally increases the likelihood of sample contamination with foreign DNA, including DNA from samples processed earlier.
a novel reagent PCRonBlood™ capable of effectively neutralizing the substances in human blood that inhibit DNA amplification. Use of PCRonBlood™ for Human Blood enables direct amplification of DNA from Human blood.

Features of PCRonBlood™

- DNA present in blood can be amplified directly.
- No DNA extraction is required
- Only small volumes of blood are necessary for the procedure.
- Risk of cross contamination and/or procedural error is dramatically reduced.
- Three types of anticoagulants (sodium citrate, dipotassium EDTA and sodium heparinate) are acceptable.
- Blood (citrated or EDTA-treated blood is recommended) stored frozen for long periods can be analyzed.
- Easy PCR method permits mass screening and routine use in a broad field of applications.

Protocol for PCRonBlood™ of Human Blood[1]

Prepare the PCR reaction mixture with
5x PCRonBlood™-Hu
(Addition of 5x PCRonBlood™ Enhancer-Hu may be useful)
↓
Aliquot PCR reaction mixture to each PCR tube
↓
Add Human Blood to each tube[2,3]
↓
Overlay mineral oil, if needed
↓
PCR[4-7]

Preparation of the PCR Reaction Mixture

5xPCRonBlood™-Hu	10 µl	4 µl
(5xPCRonBlood™ Enhancer-Hu)	10 µl	4 µl)
dNTP (5 mM each)	2 µl	0.8 µl
5'-Primer[8]	0.5 µM	0.5 µM
3'-Primer [8]	0.5 µM	0.5 µM
Taq DNA Pol (5 U/µl)[8,9]	0.5 µl	0.25 µl
Distilled water	to 50 µl	20 µl

1. We recommend that these procedures be carried out on ice (except when Hot Start PCR is used) to avoid any non-specific reactions and/or reduced sensitivity.

2. We recommend the use of 1.0µl human blood in 50µl reaction volume or 0.5µl in 20µl reaction volume, as excess blood may in some cases reduce efficiency, depending on the individual blood samples and primer sequences used.

3. Any agitation should be strictly avoided.

4. Temperature profile for direct PCR of human blood:

Pre-heating at	80°C for 15 min[5]
	94°C for 4.5 min
Followed by 40 cycles[6]	
	94°C for 30 sec
Annealing Temperature[7]	for 1 min
	72°C for 1 min
Finally	72°C for 7 min

5. Pre-heating at 80°C for 15 min is useful when fresh blood is used.

6. Five more PCR cycles are required than for purified DNA.

7. We recommend decreasing annealing temperature in 1°C increments, when poor PCR product is detected.

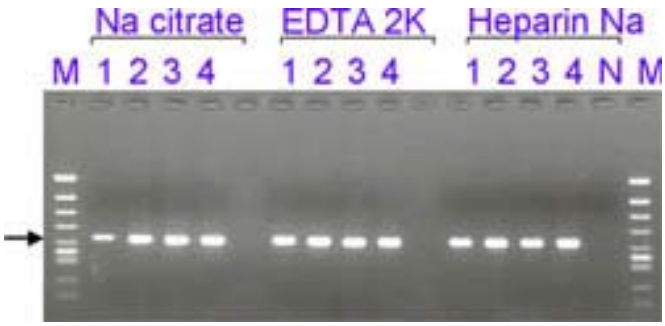
8. Not Included in this version of PCRonBlood™

9. PCRonBlood™ is effective for *Taq* DNA Polymerase with the exception of polymerase activated chemically by higher temperature (e.g. AmpliTaq Gold, Applied Biosystems, and HotStar Taq DNA Polymerase, Qiagen). However, *Taq* DNA Polymerase plus anti-*Taq* antibody permits hot start direct PCR.

Experimental Result

PCRonBlood™ on human blood treated with three different types of anticoagulants (sodium citrate, dipotassium EDTA and sodium heparinate) was examined. Human blood (0.68µl-5.0µl) treated with each anticoagulant was added to PCR reaction mixture (final 50µl) prepared using PCRonBlood™ for Human Blood or Standard Buffer⊗. The target sequence (β-globin: 408 bp) was then amplified directly.

⊗ 10mM tris-HCl, pH 8.3, 50mM KCl and 1.5mM $MgCl_2$



LaneM: DNA marker
Lanes 1-4, for each anticoagulant:
direct amplification of human blood. Respective volumes of blood within each set were 5.0µl (Lane 1), 2.5µl (Lane 2), 1.25µl (Lane 3) and 0.68µl (Lane 4)
Lane N: Negative Control

PCR was performed at 94°C for 4.5 min, followed by 40 cycles of amplification (94°C for 30 s, 55°C for 1 min, 72°C for 1 min) followed by final extension (72°C for 7 min.)

Related Products

Description	Cat. No.
Taq DNA Polymerase (500 Units) *with 10X Ammonium Reaction Buffer* *with 10X Standard Reaction Buffer*	**110303**
Taq DNA Polymerase (500 Units) *with 10X Combination Buffer*	**110403**
Taq DNA Polymerase (500 Units) *with 10X Mg^{++} Free Ammonium Buffer*	**110503**
Taq DNA Polymerase 2.0X Master Mix (100 Reac) *with 2.0 mM MgCl2*	**150301**
Taq DNA Polymerase 2,0X MaMi RED (100 Reac) *with 1.5 mM MgCl2,*	**180301**
Taq DNA Polymerase 2.0X MaMi RED (100 Reac) *with 2.0 mM MgCl2*	**190301**
AccuPOL DNA Polymerase (500 Units)	**210303**
TEMPase Hot Start DNA Polymerase (500Units) *with 10X TEMPase Buffer I* *with 10X TEMPase Buffer II*	**220303**
UniPOL –Long Range PCR (100 Reac)	**270701**
Rapid Ligation Kit (50 React)	**750300**
RT-PCR One Tube (100 Reac)	**740301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer I* (100 Reac)	**230301**
TEMPase Hot Start 2X Master Mix *with TEMPase Buffer II* (100 Reac)	**230701**
dNTP Mix (2 x 500µl) (12.5 mM of each dA, dC, dG and dT)	**501004**
dNTP Mix, (2 x 500 µl) (10 mM of each dA, dC, dG and dT),	**502004**
GC5 Value Efficiency, 10^8 Cfu/µg pUC19 Chemically Competent Cells, (10x 200µl)	**812010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (10x 50µl)	**805010**
GC5 High Efficiency, 10^9 Cfu/µg pUC19 Chemically Competent Cells, (5x 200µl)	**802005**
SuperPath GC10, 10^{10} Cfu/µg pUC19 ElectroCompetent Cells, (5x 80µl)	**830805**
SOC Medium, 10x 10mL	**800000**

NOTICE
In certain countries, patents cover the PCR process. This product is intended for researchers having a license to perform PCR or those not required to obtain a license.