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Blood Taq DNA polymerase

(Catalogue number B222, B223, B224) rev. 01/2022

Description

Blood Taq DNA polymerase is a novel genetically engineered version of Taq DNA polymerase for hot start PCR. Multiple mutations make the enzyme resistant to the inhibitory effects of blood inhibitors, DNA intercalating dyes and some other PCR inhibitors. The enzyme typically remains functional in the presence of up to 20% whole blood where other commercial enzymes fail. Resistance to blood inhibitors and higher temperatures allows to perform PCR from blood samples in the absence of DNA isolation. This property dramatically simplifies PCR preparation. Hot start PCR is secured by the presence of DNA aptamer, which binds to Blood Taq DNA polymerase and reduces production of nonspecific DNA fragments

Blood Taq DNA polymerase

This new mutant form of Taq DNA polymerase catalyzes synthesis of complementary DNA strand in the 5'->3' direction but does not possesses a 5'->3' exonuclease activity. The mutations resulted in enhanced processivity and tolerance to various inhibitors. The major usage of the enzyme is for routine diagnostic analysis for amplification of DNA fragments up to 5000 bps from multiple DNA template sources, especially from whole blood and other difficult-to-amplify samples.

Hot start

This product contains DNA aptamer, which binds specifically to Blood Taq DNA polymerase and blocks its enzymatic activity. At temperature over 50°C the aptamer is released and enzymatic activity of DNA polymerase is restored. This decreases formation of nonspecific DNA fragments during PCR. When compared to anti-Taq monoclonal antibody, which is widely used for hot start PCR, the aptamer has several advantages: (1) it is not denatured at temperature up to 100°C, (2) it has relatively low molecular weight, (3) it has defined composition, (4) it is produced synthetically, (5) it is not contaminated with components of mammalian cells, (6) it is resistant to the action of proteases and therefore allows preparation of PCR mixes and sending the enzyme without cooling, and (7) it is less expensive.

Technical data

Components and packaging

- Blood Taq DNA polymerase is supplied at a concentration 1 U/µl. Basic packaging contains 1 tube with 500 U/500 µl (B222), 5 tubes with 500 U/500 µl (B223) or 10 tubes with 500 U/500 µl (B224).
- Each tube of Blood Taq DNA polymerase is accompanied with a tube (1.5 ml) containing 10x concentrated PCR Blue buffer with 25 mM $MgCl_2$. If different concentration of $MgCl_2$ is required, a tube with 10x concentrated PCR Blue Buffer without $MgCl_2$ (1.5 ml) and a tube with 25 mM $MgCl_2$ (0.5 ml) should be ordered (Cat. No. T059).

Storage

- Long-term, at temperature -20°C ± 5°C. For short time (days) it is possible to store it at temperature up to 30°C. This allows its transport at ambient temperature.

Composition

- Storage buffer: 20 mM Tris-HCl (pH 8.0 at 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40, 0.5% Tween 20, 50% glycerol.
- 10x PCR blue reaction buffer: 750 mM Tris-HCl, pH 8.8 (at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 25 mM $MgCl_2$.

Activity

- One unit is defined as the amount of enzyme, which catalyzes incorporation of 10 nmol dNTPs within 30 min at 72°C into trichloracetic acid precipitable denatured DNA. Reaction conditions are as follow: 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM [α-^{32}P]dTTP, 50 µg/ml denatured cDNA, 0.5 µM primer and 0.2 – 0.5 U of enzyme in the volume of 50 µl.

Quality control

- Purity of Blood Taq DNA polymerase is tested by SDS-PAGE. Enzyme migrates as a single band of about 62 kDa. Material is nuclease free.
- Each batch of Blood Taq DNA polymerase is tested for its ability to amplify DNA fragment from mammalian genomic DNA in whole blood (final concentration of blood in PCR mixture is 10%). The final products are analyzed by electrophoresis in agarose gel in the presence of ethidium bromide; DNA band of the expected size is dominant (Fig. 1 and 2).

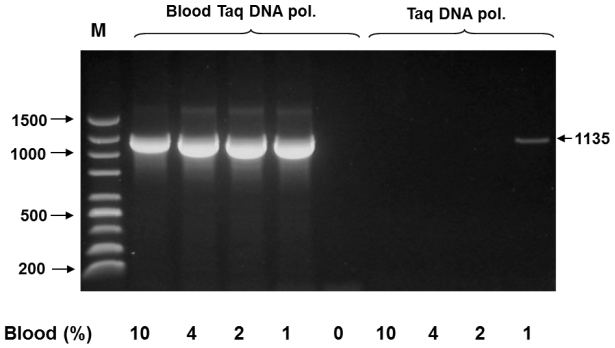

Fig**. 1.** Amplification of DNA fragment of human CCR5 gene using PCR with unseparated blood collected into Na citrate. Final blood concentration in PCR mixture was 10%, 4%, 2%, 1%, or 0%. For amplification Blood Taq DNA polymerase (Top-bio, Cat. No. B222; 2,5 U/50 µl) or generally used Taq DNA polymerase (Top-Bio Cat. No. T111; 2,5 U/50 µl) were used. PCR mixture contained 1x Blue buffer with 2.5 mM $MgCl_2$ (Cat. No. T058), PCR dNTP mix (each nucleotide at 200 µM; Cat. No. P041), 500 nM primers, and the corresponding amount of blood. For amplification, the following cycling conditions were used: initial denaturation (5 min at 95°C) 30 cycles involving denaturation (15 s at 94°C), primers annealing (15 s at 55°C) and DNA synthesis (45 s at 72°C), and at the end there was final extension (7 min at 72°C). PCR products were mixed with loading buffer (Red load, Cat. No. P066), analyzed by electrophoresis in 1% agarose gel (Cat. No. P045) a visualized by staining with ethidium bromide (Cat. No. P046).

Conclusion: The results show that Blood Taq DNA polymerase is capable to amplify 1135 bp fragments of genomic DNA from nonseparated blood at the final concentration up to 10%. Regular Taq DNA polymerase is capable to amplify the same fragment from unseparated blood at 1% concentration; higher concentrations are inhibitory.

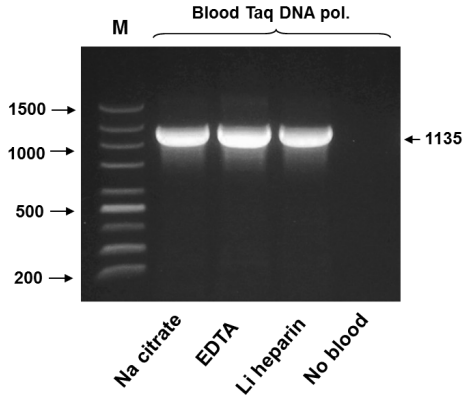

Fig. 2. Amplification of DNA fragment of human CCR5 using PCR and nonseparated blood with various anticoagulants. Human blood was collected into 3.2% Na citrate (BD Vacutainer – Na citrate; 9NC), EDTA (BD Vacutainer – EDTA; K2E) or 17 IU-ml lithium heparin (BD Vacutainer – Li heparin; LH-BD PSTTM; polymer, gel). Final concentration of blood was 10%. For amplification, Blood Taq DNA polymerase (Top-Bio; Cat. No. B2222) was used. Composition of PCR mixtures, cycling conditions and evaluation of PCR amplicons production is described in Fig. 1 legend.

Conclusion: The results show that Blood Taq DNA polymerase is capable to amplify fragments of genomic DNA of 1135 bp from 10% whole blood, not depending on anticoagulant used.

Protocol

The protocol described below can be used for routine PCR with Blood Taq DNA polymerase. However, in some cases reaction conditions must be optimized, mainly annealing temperature and concentration of $MgCl_2$. When whole blood is used as a source of DNA template, initial denaturation at 95°C for 5 min is required.

1. In the thin-wall test tube the following components are mixed[1]:

	PCR in 50 µl	**Final concentration**
10x reaction Blue buffer with 25 mM $MgCl_2$[2]	5 µl	1x react. buffer with 1.5 mM $MgCl_2$
PCR dNTP mix (10 mM each; Cat. No. P041)	1 µl	0.2 mM,each dNTP
5´ primer (50 µM)	0.5 µl	0.5 µM
3´ primer (50 µM)	0.5 µl	0.5 µM
Blood Taq DNA polymerase (1U/µl)	2.5 µl[3]	2.5 U (0.05 U/µl)
Template DNA (nonseparated blood)	x µl	Differs
PCR H_2O (Cat. No. P042)	To final vol. 50 µl	

[1] When more DNA samples are tested with the same primer set , it is convenient to prepare Master Mix in which the volumes of individual components (without template DNA) are multiplied by the number of DNA samples tested. Then, e.g. 45 µl aliquots of the Master Mix are distributed into the PCR tubes, followed by addition of the tested DNA (e.g. 5 µl of nonseparated blood) into each tube.
[2] If unsatisfactory results are obtained, PCRs with different concentrations of $MgCl_2$ should be performed (see below).
[3] To determine optimal enzyme concentration, enzyme titration should be performed.

2. Samples are homogenized and spun down.
3. If cycler without heating lid is used, PCR oil 25 µl (Cat. No. P043) is added.
4. Thermal cycler is programmed according to the manufacturer's instructions. A typical cycling program is:

	Temperature	**Time**	**Number of cycles**
Initial denaturation	95°C	5 min	1
Denaturation	94°C	15 s	30
Annealing of primers	55-68°C[1]	15 s	
Extension	72°C	~1 min per 1 kb	
Final extension	72°C	7 min	1
Cooling	22°C		

[1] Should be determined experimentally; usually 5°C below melting temperature of the primers.

5. After PCR, samples are mixed with loading buffer (Cat. No. P048, P062, P066, or P064) and analyzed immediately by electrophoresis in agarose gel in the presence of ethidium bromide (Cat. No. P046). Alternatively, the samples can be stored at -20°C.

Optimization of $MgCl_2$ concentration

$MgCl_2$ at 2.5 mM final concentration is suitable for most PCRs with Blue buffer. However, for some amplifications ooptimal Mg^{2+} concentration should be determined. To this end 10x conc. PCR Blue buffer without $MgCl_2$ and 25 mM $MgCl_2$ can be used (Cat. No. T059).

1. Preparation of Master Mix without $MgCl_2$ by mixing the following components:

10x PCR Blue buffer without $MgCl_2$	40 µl
PCR dNTP mix (each dNTP 10 mM)	8 µl
5´ primer (50 µM)	4 µl
3´ primer (50 µM)	4 µl
Blood Taq DNA polymerase (1U/µl)	20 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H_2O	236 µl
Total volume	**320 µl**

2. Master mix is mixed, centrifuged and 40 µl aliquots are distributed into 7 PCR tubes.

3. $MgCl_2$ and PCR H_2O are added into the 40 µl aliquots PCR Master mixes as follows:

Tube No.	**25 mM $MgCl_2$**	**PCR H_2O**	**Final $MgCl_2$ concentration**
1	2 µl	8 µl	1.0 mM
2	3 µl	7 µl	1.5 mM
3	4 µl	6 µl	2.0 mM
4	5 µl	5 µl	2.5 mM
5	6 µl	4 µl	3.0 mM
6	8 µl	2 µl	4.0 mM
7	10 µl	0 µl	5.0 mM

4. PCR is performed as above and the samples are analyzed by electrophoresis in agarose gel in the presence of ethidium bromide. Optimal concentration of $MgCl_2$ for given PCR is thus determined.

Cat. No.	Product name and specification	Amount
B222	Blood Taq DNA polymerase	500 U
B223	Blood Taq DNA polymerase	5x 500 U
B224	Blood Taq DNA polymerase	10x 500 U
T059	10x conc. PCR Blue buffer without $MgCl_2$+$MgCl_2$	1.5 ml + 0.5 ml