KAPA Blood PCR Kit

Kit codes and components	
KK7001 Sample Kit containing 2 x 25 reactions	KAPA Blood PCR Mix A (2x) 1 x (25 x 25 µl rxns) KAPA Blood PCR Mix B (2x) 1x (25 x 25 µl rxns)
KK7002 500 reactions	KAPA Blood PCR Mix **A** (2x) 5 x (100 x 25 µl rxns)
KK7003 500 reactions	KAPA Blood PCR Mix **B** (2x) 5 x (100 x 25 µl rxns)
KK7004 1,000 reactions	KAPA Blood PCR Mix **A** (2x) 10 x (100 x 25 µl rxns)
KK7005 1,000 reactions	KAPA Blood PCR Mix **B** (2x) 10 x (100 x 25 µl rxns)

1. Product Description

The KAPA Blood PCR Kit is designed for the amplification of DNA fragments directly from whole blood. The product is based on KAPA Blood DNA Polymerase, a second-generation enzyme derived through a process of molecular evolution and the first DNA polymerase engineered specifically for Whole Blood PCR. The enzyme is available in KAPA Blood PCR Mix A or B, two optimized, easy-to-use 2x formats containing all PCR components except primers and template (whole blood).

2. Applications

KAPA Blood PCR Kits have been validated for the direct amplification of DNA fragments from whole blood collected in EDTA anticoagulant tubes, on FTA® Elute Cards, Whatman 903® Specimen Collection Paper ("Guthrie cards") or regular filter paper. Positive results have been obtained with blood stored in EDTA tubes at 4°C for >2 years, as well as with fresh and frozen blood.

DNA fragments up to 3.5 kb (with a GC content <65%) have been amplified successfully using KAPA Blood PCR Kits. The amplification of fragments with a high GC content (>65%) is possible in the presence of DMSO, but the maximum amplicon length is limited to ≤1 kb.

- **Sample types:** The product is **not suitable** for the amplification of DNA from blood collected in anticoagulant tubes containing heparin. It has not been validated for direct amplification from blood collected in other anticoagulant tubes (e.g. citrate, ACD). Positive results with other crude sample types, including buccal swabs and amniotic fluid, have been reported. KAPA2G Robust HotStart PCR kits are recommended for direct amplification from other crude sample types.
- **Species:** KAPA Blood PCR Kits have primarily been validated in Whole Blood PCR using **human** blood, but may also be used with blood from other species collected in a suitable manner. Positive results with blood from other mammals (mice, cats) and several bird species have been reported. The optimal concentration of blood in a KAPA Blood PCR must be determined empirically for each species. Blood from species with nucleated erythrocytes typically have to be pre-diluted to more closely approximate the DNA concentration present in the same volume of human blood.
- **Applications:** DNA fragments derived from pathogens have been successfully amplified from suitable blood samples using KAPA Blood PCR Kits. However, the product has not been validated for pathogen detection and is currently recommended for **genetic testing**. Whole Blood PCR with this product is a viable alternative to amplification of DNA purified from blood (using crude extraction methods or DNA purification kits) for the following applications:
  - Single amplicon or multiplex end-point PCR, using unlabelled or fluorescently labelled primers.
  - SNP analysis, based on restriction endonuclease digestion of PCR products.
  - Paternity testing using the PowerPlex® 16 System (Promega Corporation).
- The product has not been validated for real-time/quantitative PCR applications using SYBR® Green or related dyes or sequence-specific probes (e.g. TaqMan®, Molecular Beacons).

Storage

Store all components at –20°C for long term use. For short term use (≤1 week), 2x KAPA Blood PCR Mixes may be stored at 4°C.

Quick Notes

- KAPA Blood PCR Mixes (2x) contain all components required for Whole Blood PCR, except primers and template (blood).
- Use KAPA Blood PCR **Mix A** (2x) for assays employing sensitive fluorescent detection systems (e.g. for paternity testing using the Promega PowerPlex® 16 system).
- Use KAPA Blood PCR **Mix B** (2x) for GC rich amplicons, other difficult amplicons and assays based on analysis by agarose gel electrophoresis/ethidium bromide staining.
- Amplify DNA fragments directly from blood collected in EDTA anticoagulant tubes, on FTA® Elute Cards, Whatman 903® paper ("Guthrie cards") or regular filter paper.
- Not suitable for direct amplification from blood collected in anticoagulant tubes containing heparin.
- For amplification from human EDTA blood, use 1 - 20% v/v blood in the PCR.
- For GC-rich amplicons, include 5% DMSO in reactions.
- Spin PCR products to collect debris prior to analysis.
- Purification of PCR products with a standard PCR cleanup kit prior to RE digestion, DNA sequencing or dHPLC analysis is recommended.

3. Reaction setup

3.1 Typical reaction setup using EDTA blood:

Whole human EDTA blood may be added to a final volume of 1 - 20% in the PCR reaction (i.e. 0.5 - 10.0 µl in a 50 µl reaction). Reaction volumes ranging from 10 to 50 µl may be used. Thorough mixing of the blood and other reaction components prior to thermal cycling is important.

A typical reaction is set up by mixing the components in the order listed in the table below. Once pipetting has been completed, shake or spin tubes briefly to collect all components in the bottom of the tube. Vortex to mix but do not spin again before reactions are placed in the thermocycler.

Reaction component	Final concentration	Per 50 µl rxn	Per 25 µl rxn	Per 10 µl rxn
PCR grade water		Up to 50.0 µl	Up to 25.0 µl	Up to 10.0 µl
Forward primer (5 µM)[1]	0.25 µM	2.50 µl	1.25 µl	0.50 µl
Reverse primer (5 µM)[1]	0.25 µM	2.50 µl	1.25 µl	0.50 µl
DMSO (100%) or Tween 20® (2%)[2]	(5% or 0.1% v/v)	(2.50 µl)	(1.25 µl)	(0.50 µl)
KAPA Blood PCR Mix A or B (2x)	1x	25.0 µl	12.5 µl	5.00 µl
EDTA blood[3]	10% v/v	5.0 µl	2.50 µl	1.0 µl

[1]To facilitate reaction setup, a primer premix containing 5 µM of each primer may be used and the volumes of primer and PCR grade water added to each reaction adjusted accordingly. For multiplex PCR, use a 20x primer premix containing each set of forward and reverse primers at the optimal concentration. If a commercial primer premix is used, use the final concentration of each primer recommended by the supplier.

[2] Include DMSO for amplicons with a GC content >65% that fail to amplify in the absence of DMSO. Some amplicons with a GC content <65% may also benefit from the inclusion of DMSO in the reaction. Yields of long amplicons may be improved by including Tween 20® in the reaction to a final concentration of 0.1% (v/v).

[3]Use a final concentration of 10% v/v human blood as a first approach. For longer or GC-rich amplicons, more (up to 20% v/v) or less (as little as 1% v/v) may yield optimal results. For other species, the optimal concentration of blood in a reaction must be determined empirically.

3.2 Typical reaction setup using blood collected on FTA® Elute Cards , "Guthrie cards" or filter paper:

- Use the same reaction setup as above, but replace the volume of EDTA blood with PCR water.
- Pipette reaction components (except blood) in the order listed in the table above; shake or spin tubes briefly to collect components in the bottom of each tube.
- Punch a disc from each sample card or filter paper and dispense directly into the appropriate reaction tube. The diameter of the disc may be adjusted according to the reaction volume and amplicon type (use larger discs for larger reaction volumes, long and GC-rich amplicons). **Make sure not to cross-contaminate samples when discs are punched.**
- Vortex reaction tubes and place in the thermocycler.

3.3 Important considerations:

- Reaction setup at room temperature is possible due to the intrinsic hot start nature of Whole Blood PCR – template DNA is only released when hematocytes are lysed during the initial denaturation step. However, the enzyme is active at room temperature and it is recommended that primers be designed carefully to eliminate primer-dimer formation at low temperatures.
- If multiple samples are interrogated with the same primer set, a master mix containing all of the components listed above, except template (blood or sample discs) may be prepared to reduce pipetting steps. Mix components thoroughly by vortexing. The master mix should be kept on ice, but can be kept at room temperature during aliquotting for up to 1 hour. Do not store master mixes for use at a later stage.
- If reactions are set up at room temperature, ensure that reaction mixes that contain blood or sample discs are not kept at room temperature for more than 30 min prior to thermal cycling. If needed, completed reactions may be kept on ice for up to 2 hours before cycling is performed.
- Primer quality plays an important role in the efficiency of Whole Blood PCRs. For best yields and specificity of amplification, dilute primers in 10 mM Tris-Cl, pH 8.5 and avoid using primers that have undergone multiple freeze-thaw cycles.

4. Cycling parameters

Best results are obtained when Whole Blood PCR is performed with the same cycling profile optimized for the assay using isolated DNA as template, especially when the original assay uses a "touchdown" protocol. However, make sure that:

- The initial denaturation time is 5 - 10 min (94 - 95°C).
- The denaturation time in each cycle is at least 30 sec (94 - 95°C).
- The annealing time in each cycle is at least 30 sec (use the same annealing temperature as for the original assay).
- The extension time in each cycle is at least 1 min/kb at 72°C.

Use the same number of cycles as in the original protocol. A final extension step is not strictly required, but 1 min per kb at 72°C may be included.

A typical 3-step cycling profile for Whole Blood PCR with KAPA Blood PCR Mix A or B is given in the table below.

Cycling step	Time & temperature
Initial denaturation	5 min at 95°C
Denaturation	30 sec at 95°C
Annealing	30 sec at optimal Ta
Extension	1 min/kb at 72°C
No. of cycles	30 - 40
Final extension	0 - 1 min/kb at 72°C

5. Post-PCR processing and analysis

Using whole blood instead of DNA as the template in a PCR may have implications for downstream processing and analysis. During Whole Blood PCR, proteins and other organic debris are released from hematocytes and are present in the PCR product together with the amplified DNA. A significant fraction of these compounds may be eliminated by centrifugation of the PCR product. However, the cleared supernatant may still contain salts and other compounds that are inhibitory to some downstream processes and analysis methods.

- **Spin KAPA Blood PCR products for at least 5 min at maximum speed (14,000 - 17,000 x *g*)** in a benchtop microcentrifuge prior to post-PCR processing or analysis. If PCRs were performed in plates, centrifugation times may have to be increased significantly to compensate for the lower *g*-force limits of microplate centrifuges. To obtain the most compact pellet of organic debris (and facilitate recovery of the amplicon-containing supernatant), avoid handling of PCR products in a manner that may result in the distribution of debris across the inside surface of the tube or plate (e.g. do not invert tubes or plates).
- **DNA Sequencing and/or analysis by fluorescent capillary electrophoresis:** Carefully remove the cleared supernatant, process and analyze using standard protocols for PCR products generated with isolated DNA as template. Purification of the cleared supernatant with a standard PCR cleanup kit prior to DNA sequencing is strongly recommended.
- **dHPLC analysis:** KAPA Blood PCR Mixes have been formulated not to contain any compounds that are refractory to analysis of PCR products by dHPLC (see table on next page). However, the organic debris (particularly denatured protein) present in Whole Blood PCR products, may adversely affect dHPLC systems. Purification of KAPA Blood PCR products with any standard PCR cleanup kit is therefore recommended prior to dHPLC analysis.
- **RFLP analysis:** Some restriction endonucleases will function fully in the cleared supernatants of KAPA Blood PCR products, whereas others will yield incomplete digests or will not digest at all. Whether or not a specific RE may be used in a direct post-PCR digest depends primarily on the activity requirements of the RE and has to be determined empirically. If incomplete digestion is observed, cleared supernatants should be purified with a standard PCR cleanup kit prior to RE digestion.

For advanced troubleshooting options or assistance with reaction optimization,
e-mail **support@kapabiosystems.com** or visit **http://www.kapabiosystems.com**

dHPLC compatibility of KAPA Blood PCR Mixes A and B

Compound	Maximum final conc. in PCR product	Concentration in 1x KAPA Blood PCR Mix*
Mineral oil, formamide, Proteinase K, BSA	None	Not present
High molecular weight stabilizers (e.g. PEG)	<1%	Not present or ≤1%
Detergents (e.g. SDS, Triton® X-100, Tween-20®, Nonidet®-P40)	<1%	Not present or ≤1%
Glycerol	2%	Not present or ≤2%
Betaine	2.5 M	Not present or ≤2.5 M
DMSO	10%	Not present or ≤10%

*Values apply to the components contained in KAPA Blood PCR Mix A or B **only** and not to any components derived from whole blood or any other sample type added as template in the PCR.

6. Specifications

6.1 Shipping and storage

KAPA Blood PCR Kits are shipped on ice packs. Upon receipt, store the entire kit at -20ºC in a constant temperature freezer. When stored under these conditions and handled correctly, all kit components will retain full activity for 6 months from date of receipt.

6.2 Handling

Depending on the actual temperature of the freezer in which KAPA Blood PCR Kits are stored, the PCR Mix may or may not freeze. If frozen, thaw fully before use. Avoid more than 10 freeze-thaw cycles of any individual tube. For short term usage (≤1 week) individual tubes may be stored at 4°C. Always vortex KAPA Blood PCR Mixes before use to ensure that components are thoroughly mixed.

6.3 Quality control

KAPA Blood DNA Polymerase is extensively purified through the use of multiple chromatography steps. The final formulation contains <2% contaminating protein, as determined in an Agilent Protein 230 Assay. Each batch of KAPA Blood PCR Mix is subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

6.4 Product use limitations and licenses

KAPA Blood PCR Kits are developed, designed and sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual component, was tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, which is available on request.

Certain applications of this product are covered by patents issued to parties other than Kapa Biosystems and applicable in certain countries. Purchase of this product does not include a license to perform any such applications. Users of this product may therefore be required to obtain a patent license depending upon the particular application and country in which the product is used.

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