Current workflows for the extraction and amplification of DNA from blood samples for PCR-based testing can benefit from improvements in turnaround time and reliability.

KAPA Express Extract, combined with KAPA2G Robust HotStart ReadyMix, is ideally suited for the routine extraction and amplification of DNA from blood samples for PCR testing. The novel KAPA Express Extract kit offers an easy and fast way to prepare PCR-ready DNA. KAPA2G Robust HotStart ReadyMix contains an engineered DNA polymerase tolerant to common PCR inhibitors, offering reliable amplification of DNA extracted from blood samples.

Introduction

PCR-based testing and genetic testing is a well-established and fast growing discipline. Blood, collected in anticoagulant tubes or on specimen collection cards, constitutes one of the major sample types processed for this purpose. Current workflows rely on wild-type DNA polymerases and therefore require purification of DNA from blood samples prior to amplification. The DNA isolation step represents a significant portion of the cost and turnaround time associated with PCR-based testing and poses a high risk for sample loss or contamination.

KAPA Express Extract offers a fast, convenient and efficient alternative for the routine extraction of DNA from different types of blood samples. The novel thermostable protease and buffer system has been designed for optimal cell lysis and preservation of DNA extracts. The KAPA Express Extract enzyme and buffer are combined in a single tube with EDTA blood (2 – 8 µL), or a punch from a blood collection card (1 – 2 mm diameter). Lysis is performed in a standard thermocycler, after which the reaction product is centrifuged and the DNA-containing supernatant recovered. This protocol eliminates excessive handling, thereby reducing the risk of sample contamination or loss and generates PCR-ready DNA in as little as 15 min. The process produces sufficient template for multiple assays is easily scaled to handle multiple samples in a 96-well format.

KAPA2G Robust Hotstart ReadyMix is ideally suited for the routine and reliable amplification of DNA extracted from blood samples. This ready-to-use cocktail contains KAPA2G Robust Hotstart DNA polymerase, a novel enzyme engineered for improved processivity and tolerance to common PCR inhibitors through a process of molecular evolution. The improved tolerance of the enzyme to carry-over inhibition offers more reliable routine amplification of DNA extracted from different types of blood samples. When used in combination with the KAPA Express Extract system, the turnaround times for PCR testing can be significantly reduced.

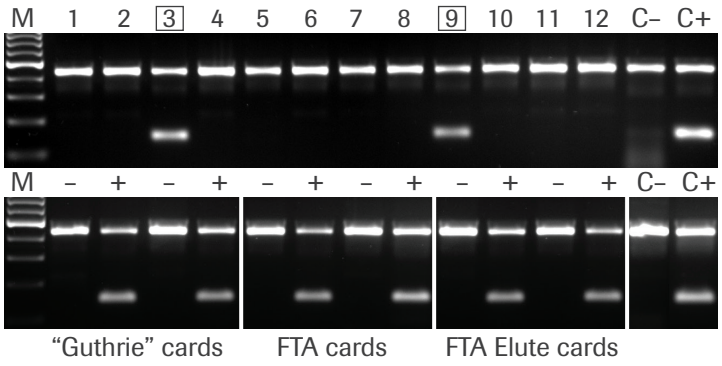

Figure 1. Extraction and amplification of DNA from different blood sample types for detection of the HLA-B*27 allele.

Top panel: DNA was extracted from 12 human EDTA blood samples with KAPA Express Extract, as outlined on the next page. Of each extract, 2 µL was used directly (without quantification) in a 25 µL PCR containing KAPA2G Robust HotStart ReadyMix and two primer sets. The internal control primer set targets a 429 bp fragment of the beta globin gene, whereas the second primer set targets a 141 bp fragment of the HLA-B*27 locus in a sequence-specific manner (Olerup, 1994). Two of the 12 individuals tested positive for the HLA-B*27 allele associated with ankylosing spondylitis. Lanes C- and C+ represent HLA-B*27 negative and positive controls, respectively (1 ng purified human genomic DNA as template).

Bottom panel: DNA was extracted from “Guthrie” cards, FTA cards or FTA Elute cards spotted with blood of individuals confirmed to be HLA-B*27 positive (+) or negative (-). DNA extraction and amplification conditions and controls (C- and C+) were the same as for the top panel.

Results

To demonstrate the suitability of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix for PCR testing from blood, DNA was extracted from four blood sample types, namely EDTA blood and blood collected on three different Whatman collection cards (903® Specimen Collection or “Guthrie” cards, FTA® cards and FTA® Elute cards) (Figure 1). Extracted DNA was used as template in a well-established HLA-B*27 assay. Samples positive for the HLA-B*27 allele were identified unequivocally from all sample types. In addition, results obtained with crudely extracted DNA were highly comparable to those generated with purified human genomic DNA.

DNA extraction protocol

An overview of the KAPA Express Extract protocol for EDTA blood or card punches is given in Table 1. Lysis reactions may be set up individually as outlined in Table 2, or a master mix containing the KAPA Express Extract enzyme and buffer may be prepared and aliquotted into individual tubes or wells containing card punches or aliquots of EDTA blood. When using EDTA blood samples, it is important to vortex the anticoagulant tube in which the blood was collected thoroughly before an aliquot is withdrawn for the lysis reaction. The amount of blood per 100 µL lysis reaction may be increased up to 10 µL to increase the yield and concentration of extracted DNA. Punches with a diameter of 1 – 2 mm are recommended for all three types of Whatman collection cards tested. After the 10 min lysis protocol, samples are centrifuged briefly to pellet debris. It is recommended that cleared lysate supernatants (70 µL per reaction) be transferred to fresh tubes or plates to limit inhibitor carry-over into subsequent PCRs. DNA extracts prepared in this way do not have to be quantified and may be used directly in a PCR. One extraction typically yields sufficient template for ~50 x 25 µL PCRs. DNA extracts may be diluted 1:5 in TE Buffer for long-term storage at -20°C.

Table 1. KAPA Express Extract protocol for blood samples

Step	Description
Reaction setup	1. Add the appropriate volumes of PCR grade water and KAPA Express Extract enzyme and buffer to each tube or well (Table 2) or 2. Prepare a bulk lysis solution by combining KAPA Express Extract Buffer, enzyme and PCR grade water, and aliquot the appropriate volume into each PCR tube or well. 3. Vortex EDTA blood tubes well and transfer 2 – 8 µL of each sample to individual PCR tubes or wells of a 96-well PCR plate or punch a 1 – 2 mm disc from each blood collection card using a sterile punch and transfer each carefully to a PCR tube or well of a 96-well PCR plate.
Lysis	1. Close tubes or seal plate and place in thermocycler. 2. Incubate at 75°C for 10 min. (During this step, cells are lysed, nucleases and proteins degraded and DNA released.)
Heat-inactivation	Incubate plate at 95°C for 5 min to inactivate the thermostable KAPA Express Extract enzyme.
Sample recovery	1. Centrifuge plate for 1 min to pellet debris. 2. Recover DNA-containing supernatant.

Table 2. KAPA Express Extract lysis reaction setup for blood samples

Reaction component	Final conc.	Per 100 µL reaction	Per 96-well plate
PCR grade water	–	Up to 100 µL	Up to 10 mL
10X KAPA Express Extract Buffer	1X	10.0 µL	1.00 mL
KAPA Express Extract Enzyme (1 U/µL)	20 mU/µL	2.00 µL	0.20 mL
EDTA blood or blood card punch	–	2 – 8 µL 1 – 2 mm	–

Table 3. KAPA2G Robust HotStart ReadyMix reaction setup for HLA-B*27 assay

Reaction component	Final conc.	Per 25 µL reaction*
PCR grade water	–	Up to 25.0 µL
2X KAPA2G Robust HotStart ReadyMix**	1X	12.5 µL
Primer premix (10 µM)†	0.25 µM (of each of 4 primers)	0.625 µL
KAPA Express Extract blood DNA extract	–	2.00 µL

*For smaller reaction volumes, scale down all volumes proportionally. Do not perform reactions >25 µL.

**Contains $MgCl_2$ at a 1X concentration of 2 mM. Additional $MgCl_2$ may be added for other assays if needed.

†A premix of the four primers described by Olerup (1994) were made to facilitate reaction setup.

Table 4. KAPA2G Robust HotStart ReadyMix cycling parameters‡

Cycling step	Temperature and time	
Initial denaturation	3 min at 95°C	
Denaturation	15 sec at 95°C	x35 cycles for EDTA blood samples
Annealing	15 sec at 60°C	
Extension	15 sec at 72°C	x40 cycles for blood card samples
Final extension	1 min at 72°C	

‡These cycling parameters are standard for KAPA2G Robust HotStart ReadyMix assays. Please refer to the **KAPA2G Robust HotStart ReadyMix Technical Data Sheet** if optimization of cycling parameters is required for other assays.

PCR reaction conditions and cycling parameters

The KAPA2G Robust HotStart ReadyMix reaction setup and cycling parameters for the HLA-B*27 assay given in Tables 3 and 4 were derived from the protocol of Olerup (1994), but adapted to the specific characteristics of the novel KAPA2G Robust enzyme. Overall cycling times were reduced considerably compared to the original protocol. With the combination of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix, reliable DNA amplification from blood samples can be achieved in ≤2 hours, compared to ≥1 day with conventional methods.

References

1. Olerup, O. (1994). *Tissue Antigens* 43: 253 – 256.

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