KAPABIOSYSTEMS

Technical Data Sheet

KAPA Express Extract Kit

KR0383 – v4.16

Product Description

KAPA Express Extract is a novel thermostable protease and buffer system that allows for the extraction of PCR-ready DNA from various tissue types in as little as 15 minutes. The KAPA Express Extract system is designed for optimal tissue lysis and sample preservation. Unlike protocols that rely on proteinase K digestion, DNA extractions with KAPA Express Extract are conveniently performed in a single tube, without the need for hazardous chemicals and multiple washing steps. This greatly reduces the risk of sample loss and contamination.

Tissue lysis is performed in a standard thermocycler, heating block, or waterbath, after which the sample is centrifuged and the DNA-containing supernatant recovered. Extracts may be used directly in PCR, without quantification or dilution. Depending on the tissue type, each extract typically yields a sufficient amount of template for 50–500 PCRs, and may be diluted in TE buffer for long-term storage at -20°C.

DNA extracted with KAPA Express Extract is compatible with any PCR system. However, KAPA2G Robust HotStart ReadyMix is recommended for the rapid and reliable endpoint amplification of DNA extracted with KAPA Express Extract. The ReadyMix contains the novel KAPA2G Robust HotStart polymerase, engineered for improved processivity and tolerance to common PCR inhibitors through a process of directed evolution. The 2X ReadyMix is a ready-to-use master mix containing all components for fast PCR, except primers and template. The combination of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix Kits enables extraction and amplification of DNA fragments from crude tissues in ≤2 hours, as compared to ≥1 day with conventional protocols.

Product Applications

KAPA Express Extract is ideally suited for the extraction of PCR-ready DNA from, but not limited to, the following sample types:

- Human tissue (FFPE samples; blood collected in EDTA tubes or on collection cards; buccal swabs; hair follicles; forensic samples)
- Animal samples (ear or tail clippings; hair follicles; blood; bone marrow; dried or fresh tissue from mouse and other mammals)
- Fish tissues (fin punches; fresh tissue; cold and hot smoked canned samples; ethanol-preserved samples)
- Insects (crushed)
- Bird feathers (calamus fragments).

Kapa/*Roche* Kit Codes and Components	
KK7100 *07961596001* (50 extractions) **KK7101** *07961600001* (100 extractions) **KK7102** *07961618001* (250 extractions) **KK7103** *07961626001* (500 extractions)	**Extraction only** 1 U/µL KAPA Express Extract Enzyme 10X KAPA Express Extract Buffer
KK7151 *07961669001* (100 reactions) **KK7152** *07961677001* (500 reactions)	**Extraction plus amplification module** 1 U/µL KAPA Express Extract Enzyme 10X KAPA Express Extract Buffer 2X KAPA2G Robust HotStart ReadyMix

Quick Notes

- Extract PCR-ready DNA from different tissue types in a quick and simple, single-tube protocol (15–20 min), without the need for hazardous chemicals or multiple washing steps.
- Significantly reduced turnaround times and risk of sample loss and contamination.
- Use 1 µL DNA extract per 25 µL PCR. Depending on the sample type, the extract may have to be diluted prior to PCR.
- One extract typically yields a sufficient amount of template for 50–500 PCRs.
- DNA extracts may be diluted in TE Buffer (1:5) or 10 mM Tris-HCl pH 8–8.5 (1:5–1:10) for long-term storage at -20°C. Extracts are stable at -20°C for ≥6 months.
- KAPA2G Robust HotStart ReadyMix is recommended for consistent and reliable endpoint amplification of DNA extracts generated with KAPA Express Extract.
- No quantification of DNA required prior to PCR.

KAPA Express Extract is **not suitable** for DNA extraction from plants; for plant PCR applications, the KAPA3G Plant PCR Kit is recommended.

Application Notes detailing the use of KAPA Express Extract are available from **www.kapabiosystems.com** for the following applications:

- Human blood PCR
- Fish DNA barcoding
- Mammalian DNA barcoding
- Human FFPE PCR
- qPCR using KAPA PROBE FORCE.

KAPA Express Extract Protocol

1. Reaction Setup

DNA extractions are performed in 100 µL volumes, and should be set up as follows:

Component	Per 100 µL reaction[1]	Final conc.
PCR-grade water	88 µL	—
10X KAPA Express Extract Buffer	10 µL	1X
1 U/µL KAPA Express Extract Enzyme	2 µL	2 U/rxn
Sample[2]	As required	—

[1] Extractions may be scaled up or down as required, but always ensure that the ratio of sample to extraction volume remains at roughly 2 mm tissue per 100 µL reaction.

[2] Refer to the table below for recommended sample sizes. Note that all sample sizes may be varied, provided that excessive amounts of material are not used.

Sample type	Size/volume
FFPE tissue	~2 mm^2 fragment of a 10 µM section, or 1 mm^3. Trim all excess wax.
Human or animal blood	2–8 µL fresh or EDTA blood; 2 mm^2 punch of blood on FTA®, FTA Elute or Guthrie cards
Hair follicle	1–10 individual follicles
Buccal swab[a]	1 swab placed directly in 300 µL reaction mixture with 0.5X KAPA Express Extract Buffer
Animal tissue	2 mm^3 fragment or 2 mm^2 punch
Insect	Place insect in tube and add reaction mixture to immerse entire insect. Crush insect with a pipette tip or similar sterile tool.
Bird feather[b]	Add 2–5 mm fragment of calamus (quill) to 50 µL reaction mixture
Fish tissue, fresh or processed	2 mm^3 fragment or 2 mm^2 punch

[a] Buccal swabs should be extracted in a volume of 300 µL, using a final concentration of 0.5X KAPA Express Extract Buffer (15 µL Express Extract Buffer, 2 µL KAPA Express Extract Enzyme, and 283 µL PCR-grade water).

[b] Feathers should be extracted in a volume of 50 µL, using 1X KAPA Express Extract Buffer and 2 U of KAPA Express Extract Enzyme per 50 µL extraction.

2. Lysis

Perform lysis in a waterbath, heating block, or thermocycler using the following protocol:

Step	Temperature	Duration
Lysis[1]	75°C	10 min
Enzyme activation	95°C	5 min

[1] Fish tissue should be used with a lysis temperature of 60°C.

NOTE: Unlike digestion with proteinase K, KAPA Express Extract does not completely degrade the tissue. There will be intact tissue visible in the tube after lysis. This does not have a negative impact on the downstream PCR.

3. Dilution

Centrifuge samples briefly to pellet cellular debris, and dilute the DNA extract 10-fold with 10 mM Tris-HCl (pH 8.0–8.5). Not all sample types require dilution, but particularly "dirty" samples such as animal tissues may benefit from a 1:10 dilution. This dilutes cellular debris and digested proteins to prevent inhibition of downstream PCR.

Extracts are stable (either diluted or undiluted) at 4°C for at least 1 month, and at -20°C for at least 6 months.

4. PCR

DNA extracts prepared with KAPA Express Extract are compatible with PCR reagents from any manufacturer. The use of the following products is recommended for best results:

- KAPA2G Robust HotStart ReadyMix: for routine end-point PCR such as genotyping or DNA barcoding with PCR fragments up to 1 kb in size. Please refer to the protocol on the next page for further details.
- KAPA2G Fast (HotStart) Genotyping Mix: for mouse genotyping and other mammalian genotyping.
- KAPA SYBR FAST: for SYBR® Green I-based qPCR.
- KAPA PROBE FAST: for probe-based qPCR. Please note that certain fluorophores may not be suitable for use with crude extracts due to fluorescence quenching. When attempting KAPA PROBE FAST, it is best to use "clean" samples, such as buccal swabs.
- KAPA PROBE FORCE: for probe-based qPCR; well-suited to crude PCR and can withstand a higher level of inhibitory material than most other qPCR mixes available.
- KAPA HiFi HotStart ReadyMix, for high-fidelity PCR. Please note that the maximum amplicon size will be substantially lower than when using purified genomic DNA.

For applications not listed here, or for advice on the most appropriate product for your application, please contact Technical Support at **kapabiosystems.com/support** for assistance.

KAPA2G Robust PCR Protocol

IMPORTANT! KAPA2G Robust HotStart ReadyMix contains an engineered DNA polymerase and uniquely-formulated buffer, and requires specialized reaction conditions. If these conditions are not adhered to, reaction failure is likely. Refer to the KAPA2G Robust HotStart ReadyMix Technical Data Sheet for additional information on important parameters.

1. Reaction Setup

- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.
- Calculate the required volume of each component based on the following table:

Component	Per 25 µL rxn[1]	Final conc.
PCR-grade water	Up to 25 µL	—
2X KAPA2G Robust HotStart ReadyMix[2]	12.5 µL	1X
10 µM Forward primer	1.25 µL	0.5 µM
10 µM Reverse primer	1.25 µL	0.5 µM
Template DNA[3]	1 µL	—

[1] Reactions may be scaled down as required. Reaction volumes >25 µL are not recommended, as reaction efficiency may be compromised.

[2] KAPA2G Robust HotStart ReadyMix contains 2 mM MgCl2 at 1X. Additional MgCl2 may be added if necessary.

[3] Use 1 µL of diluted or undiluted DNA. If yields are poor with undiluted DNA, repeat the PCR with 1:10 and 1:100 dilutions.

- Transfer the appropriate volumes of PCR master mix, template and primer to individual PCR tubes or wells of a PCR plate.
- Cap or seal individual reactions, mix and centrifuge briefly.

2. Perform PCR

- The following sample protocol is provided. Refer to the KAPA2G Robust HotStart ReadyMix Technical Data Sheet for additional information:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	35–40
Annealing	60°C	15 sec	
Extension	72°C	15–30 sec/kb	
Final extension	72°C	1 min/kb	1

Important Parameters

Lysis Reaction Composition

Extractions in 100 µL volumes are suitable for most sample types. This enables the use of PCR tubes and a thermocycler. Should larger reaction volumes be required, use an appropriate tube and incubation method (e.g., heating block or waterbath).

While precise sample size control is not necessary, do not use too much or too little sample material. The former will result in a DNA extract that contains a high concentration of inhibitors, while the latter will yield a very dilute DNA extract. In either case, a dilution series of the extract should reveal the appropriate volume to use for successful PCR. Do not exceed 5 µL of undiluted template per reaction.

Should a dilution series not result in successful PCR, the concentration of the extraction buffer may be varied between 0.5X and 2X. For some sample types, more dilute or more concentrated buffering has been shown to improve lysis efficiency.

Quantification of DNA Extracts

DNA extracts do not have to be quantified prior to use in PCR, and quantification is not recommended. Crude DNA extracts are likely to contain cellular contaminants that will affect the absorbance of the sample in the range of 260–280 nm and results in inaccurate DNA concentration determinations with spectrophotometric methods.

Furthermore, DNA extracted using KAPA Express Extract kits will be largely single-stranded due to the final enzyme deactivation step. This will yield inaccurate results with DNA quantification methods based on fluorescent intercalating dyes.

Post-lysis Processing of Samples

To pellet cellular debris, samples may be centrifuged at high speed, and the supernatant transferred to a sterile tube. Note that some samples (e.g., buccal swabs) do not contain sufficient cellular debris to form a pellet. These may be stored in the original tube. With FFPE tissue, residual wax typically collects on the sample surface during post-lysis centrifugation, and the DNA-containing liquid must be carefully removed from underneath this layer.

DNA extracts prepared with KAPA Express Extract Kits may be stored -20°C for at least 6 months, or at 4°C for up to 1 month. A 1:1 to 1:20 dilution of the DNA extract in 10 mM Tris-HCl (pH 8–8.5) or TE Buffer is recommended for long-term storage.

Product Specifications

Shipping, Storage, and Handling

KAPA Express Extract Kits are shipped on dry ice or ice packs, depending on the destination country. Upon arrival, store kit components at -20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label. KAPA2G Robust HotStart ReadyMix may not freeze solidly, even when stored at -20°C. This will not affect the shelf-life of the product. Always ensure that the product has been fully thawed and mixed before use.

Quality Control

All components contained with KAPA Express Extract Kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination.

Symptoms	Possible causes	Solutions
Nonspecific amplification or smearing	Template DNA is too concentrated	Perform a 10- to 100-fold dilution of the template, or reduce the number of cycles.
	Cycling protocol	Excessive annealing and/or extension times will result in smearing and nonspecific amplification, typically of bands larger than the target band. Reduce the annealing and extension times to the minimum recommended time.
	Annealing temperature is too low	A sub-optimal annealing temperature will typically result in nonspecific amplicons that are smaller than the target band.
	High target GC content	Supplement reactions with 5% DMSO, or use a product specifically for GC-rich PCR, such as KAPA2G Robust.
	Primer concentration	Some primers anneal more efficiently than others. Decrease the primer concentration. Store and dilute primers in a buffered solution, not water, to limit degradation.
No amplification or low yield	Template DNA concentration	Increase lysis time to a maximum of 30 min to improve release of DNA. Increase the amount of template DNA per reaction to a maximum of 5 µL of a 1:10 dilution of DNA extracted with KAPA Express Extract. Use of excessive amounts of template DNA can also limit yield by chelating Mg^{2+}, or introducing PCR inhibitors. In this case, the amount of template per reaction should be reduced, or $MgCl_2$ concentration increased in 0.5 mM increments.
	Template contains inhibitors	Repeat PCR with a 10-fold dilution series of DNA extract to determine the optimal amount of template to use per reaction. Perform positive control reactions with purified genomic DNA to confirm that the PCR protocol is optimal.
	Annealing temperature is too high	Reduce the annealing temperature by 2–5°C. Optimize the annealing temperature by gradient PCR.
	Cycling protocol	Increase the extension time. Increase the number of cycles.
	Primer concentration	Some primers anneal more efficiently than others. Increase the primer concentration, or optimize $MgCl_2$ to improve primer binding. Store and dilute primers in a buffered solution, not water, to limit degradation.
	$MgCl_2$	Optimize $MgCl_2$ concentration. AT-rich and Multiplex PCR typically require higher $MgCl_2$ concentrations.

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