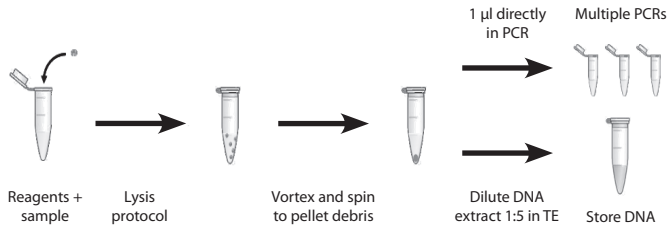
Technical Data Sheet

KAPA Express Extract

Kit code	Components
DNA Extraction Kits	
KK7100 (50 rxns)	1 x 100 U KAPA Express Extract Enzyme (1 U/µl) 1 x 1 ml KAPA Express Extract Buffer (10X)
KK7101 (100 rxns)	2 x 100 U KAPA Express Extract Enzyme (1 U/µl) 2 x 1 ml KAPA Express Extract Buffer (10X)
KK7102 (250 rxns)	1 x 500 U KAPA Express Extract Enzyme (1 U/µl) 1 x 5 ml KAPA Express Extract Buffer (10X)
KK7103 (500 rxns)	2 x 500 U KAPA Express Extract Enzyme (1 U/µl) 2 x 5 ml KAPA Express Extract Buffer (10X)
DNA Extraction Kits with PCR ReadyMix	
KK7151 (100 rxns)	2 x 100 U KAPA Express Extract Enzyme (1 U/µl) 2 x 1 ml KAPA Express Extract Buffer (10X) 1 x 1.25 ml KAPA2G Robust HotStart ReadyMix (2X)
KK7152 (500 rxns)	2 x 500 U KAPA Express Extract Enzyme (1 U/µl) 2 x 5 ml KAPA Express Extract Buffer (10X) 1 x 6.25 ml KAPA2G Robust HotStart ReadyMix (2X)

1. 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 10 min. The KAPA Express Extract system has been designed for optimal tissue lysis and sample preservation. Unlike existing protocols that rely on proteinase K digestion, KAPA Express Extractions are conveniently performed in a single-tube, without the need for hazardous chemicals and multiple washing steps. This greatly reduces the risk for sample loss and contamination. An overview of the KAPA Express Extraction protocol is given below:



Tissue lysis is performed in a standard thermocycler, after which the sample is centrifuged and the DNA-containing supernatant recovered. Extracts may be used directly in PCR, without quantification. 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 may be amplified with any PCR system. However, KAPA2G Robust HotStart ReadyMix is recommended for the routine amplification of DNA extracted using KAPA Express Extract Kits. The ReadyMix contains the novel KAPA2G Robust HotStart polymerase, engineered for improved processivity and tolerance to common PCR inhibitors through a process of molecular evolution. This allows for the reliable amplification of fragments ≤1 kb from crudely extracted DNA using a fast cycling protocol. The 2X ReadyMix is an easy-to-use cocktail containing all components needed for PCR, except for primers and template. The combination of KAPA Express Extract and KAPA2G Robust HotStart ReadyMix Kits allows for the reliable extraction and amplification of DNA fragments from crude tissues in ≤2 hours, as compared to ≥1 day with conventional protocols.

Storage, handling and specifications

Store all components at -20 °C for long-term use. Please refer to Section 6 for full details.

Quick Notes

- Extract PCR-ready DNA from different tissue types in a quick and simple, single-tube protocol (10 – 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 be diluted prior to PCR.
- No quantification of DNA prior to PCR needed.
- One extract typically yields a sufficient amount of template for 50 – 500 PCRs. DNA extracts may be diluted in TE Buffer (1:5) for long-term storage at -20 °C.
- KAPA2G Robust HotStart ReadyMix is recommended for consistent and reliable amplification of DNA extracts generated with KAPA Express Extract.

2. Applications

KAPA Express Extract Kits are ideally suited for the extraction of DNA from crude samples for most PCR applications, including qPCR analysis. However, residual inhibitors may result in quenching of commonly used fluorophores during qPCR. Depending on the sample type, a single extraction yields sufficient template DNA for 50 – 500 standard PCR reactions. For optimal results, KAPA2G Robust HotStart ReadyMix PCR Kits are recommended for downstream PCR applications. PCR-ready DNA has been successfully extracted with KAPA Express Extract Kits and amplified reliably with KAPA2G Robust HotStart ReadyMix from 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 meat 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)

3. DNA extraction protocol

The KAPA Express Extract protocol for extraction of PCR-ready DNA from various tissue samples is the following:

Step	Description	
1. Reaction setup	Combine the following in a thin-walled PCR tube and vortex to mix:	
	10X KAPA Express Extract Buffer	10 µl
	1 U/µl KAPA Express Extract Enzyme	2.0 µl (2 U)
	PCR-grade water	Up to 100 µl
	Sample	See table below
2. Lysis[1]	Incubate in a thermocycler for 10 min at 75 °C or 60 °C for fish tissues (see table below). During this step, cells are lysed, nucleases and proteins degraded and DNA released.	
3. Heat-inactivation[1]	Incubate for 5 min at 95 °C to inactivate the thermostable KAPA Express Extract protease.	
4. Sample recovery and use	Vortex reaction product for 2 – 3 sec. Centrifuge at high speed for 1 min to pellet debris. Transfer DNA-containing supernatant to a fresh tube. Use 1 µl of DNA extract directly in a 25 µl PCR, without quantification. Dilute in TE Buffer for long-term storage at -20 °C.	

[1] Programme thermocycler to perform incubations at 75 °C and 95 °C consecutively and then cool reaction products to 4 °C.

The recommended sample size and lysis protocol for different sample types are as follows:

Sample type	Sample size[1]	Lysis protocol
FFPE tissue	~2 mm^2 fragment of a 10 µm section or 1 mm^3	10 min at 75 °C 5 min at 95 °C
Human or animal blood	Fresh or EDTA blood: 2 µl. Can use up to 8 µl for higher yields. Blood on FTA®, FTA® Elute or Guthrie cards: 2 mm^2 punch	
Hair follicle	1 – 10 individual follicles	
Buccal swab[2]	1 swab placed directly in a 1.5 ml reaction tube containing 300 µl of 0.5X KAPA Express Extract Buffer	
Animal tissue	~2 mm^3 fragment or ~2 mm^2 punch	
Insect	Add 1X KAPA Express Extract Buffer to cover whole insect, crush in reaction tube and add 2 U KAPA Express Extract Enzyme	
Bird feather	Add 2 – 5 mm fragment of *calamus* (quill) to 50 µl of 1X KAPA Express Extract Buffer, add **2 U** KAPA Express Extract Enzyme	
Fish tissue (fresh or processed)	~2 mm^3 fragment or ~2 mm^2 punch	10 min at 60 °C 5 min at 95 °C

[1] All sample sizes/volumes are approximate and may be varied, provided that excessive amounts of material is not used.
[2] Because of large sample volume, the lysis protocol should be performed in a heating block and not in a thermocycler.

FTA® is a registered trademark of GE Healthcare.

4. Important parameters

4. 1 Extraction reaction setup and optimization of DNA output

- **Reaction volume and final KAPA Express Extract Buffer concentration:** 100 µl reactions, containing KAPA Express Extract Buffer at a final concentration of 1X, are recommended for most sample types, except for buccal swabs which are lysed in 0.5X KAPA Express Extract Buffer in 1.5 ml reaction tubes. For samples that are difficult to lyse or prone to yielding degraded DNA, the final concentration of the KAPA Express Extract Buffer may be varied from 0.25X to 2X. The buffer may be diluted with PCR-grade water.
- **Lysis and heat-inactivation:** The lysis step at 75 °C may be varied between 10 and 30 min for different sample types. Incubation times <10 min may result in inadequate lysis and low DNA yields, whereas incubation times >30 min may result in DNA damage. For fish tissues, optimal results are achieved by performing the lysis step at 60 °C instead of 75 °C. Heat-inactivation of the KAPA Express Extract enzyme for at least 5 min at 95 °C is essential as carryover enzyme will degrade the DNA polymerases during PCR.
- **Recovery of DNA-containing extract after lysis and enzyme de-activation:** The ease of DNA recovery is dependent on the sample type. In many cases, centrifugation of the reaction product for 1 min at high speed is sufficient to pellet cellular debris, and the supernatant is easily recovered. In some cases, debris does not pellet but remains suspended throughout the reaction product. In such cases, it is recommended that the DNA-containing liquid be carefully transferred to a fresh tube for downstream use and storage. Do not try to recover the entire volume at once, but rather collect smaller volumes at a time, and change the pipette tip regularly to minimize carryover of debris. 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. To facilitate sample recovery from FFPE tissues, it is important to trim all excess wax from the tissue before transferring it into the lysis reaction.
- **Contamination control:** To minimize the risk of sample contamination, it is recommended that extraction reactions and downstream PCRs are set up in a PCR hood.
- **Long-term storage of DNA extracts:** DNA extracts generated with KAPA Express Extract Kits may be stored -20 °C for use in multiple PCRs over a period of time. A 1:5 dilution of the DNA extract in TE Buffer is recommended for long-term storage. This is done to ensure that the DNA is stored in a buffered environment. However, the dilution factor may be varied between 1:1 and 1:20, depending on the downstream application and yield of DNA. For downstream applications that are sensitive to EDTA, TE may be replaced with 10 mM Tris-HCl, pH 8.0 − 8.5. DNA extracts stored in this manner are typically stable for 6 months.

4. 2 Amplification of DNA extracted with KAPA Express Extract Kits

- **Recommended product:** KAPA2G Robust HotStart ReadyMix Kits or KAPA2G Robust HotStart PCR Kits are recommended for the routine and reliable amplification of DNA fragments ≤1 kb from crude extracts generated with KAPA Express Extract Kits. KAPA2G Robust is a second-generation DNA polymerase, engineered for improved processivity and tolerance to common PCR inhibitors. This ensures reliable amplification of crudely extracted DNA, even in the presence of residual inhibitors. In addition, a fast cycling protocol may be used, thereby further reducing the turnaround time from sample preparation to final result.
- **Reaction setup and cycling parameters:** Please refer to the KAPA2G Robust HotStart Technical Data Sheet for details regarding PCR setup and cycling conditions. Use 1 µl of DNA extract in a 25 µl KAPA2G Robust HotStart PCR as a first approach. Depending on the sample type, the volume of DNA extract used may be increased to a maximum of 5 µl per 25 µl PCR. Extracts with a high template concentration may be diluted 1:10 to 1:100 prior to PCR. It is important that the recommended cycling parameters are followed. Start with 15 sec annealing at 60 °C and 15 sec extension at 72 °C and perform 40 cycles. To improve yields, the extension time may be extended to 30 or 45 sec per cycle, but the annealing time should never be increased. The annealing temperature may be varied between 55 and 65 °C, depending on the properties of the primers used. If smearing occurs, reduce the number of cycles, increase the annealing temperature or reduce the extension time to 5 – 10 sec per cycle.
- **Inhibitor carryover:** Some crude DNA extracts may contain high concentrations of PCR inhibitors. If the recommended cycling parameters fail to yield acceptable results, it may be necessary to dilute the DNA extract prior to PCR. Repeat the experiment with a positive control sample (e.g. genomic DNA purified using a column-based kit) and with a 10-fold dilution series of the DNA extract (prepared in TE or 10 mM Tris-HCl, pH 8.0 − 8.5). The DNA extracts may also be "spiked" with a known concentration of the positive control sample to determine whether the inhibitors may be diluted out, whilst retaining a high enough template concentration to support amplification of the target. DNA extracts generated with KAPA Express Extract Kits may be used in qPCR analysis; however, residual inhibitors may result in quenching of commonly used fluorophores during qPCR. The degree of quenching is dependent on the sample type and the fluorophore used. Dilution of the DNA extract with TE or 10 mM Tris-HCl, pH 8.0 − 8.5 lysis may yield acceptable results. Other solutions include using an alternative fluorophore, or purifying the DNA further by ethanol or isopropanol precipitation or a DNA clean-up column.
- **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 result in inaccurate DNA concentration determinations based on spectrophotometric methods. Furthermore, DNA extracted using KAPA Express Extract Kits will be largely single-stranded due to the final enzyme de-activation step. This will yield inaccurate results with DNA quantification methods based on fluorescent intercalating dyes.

5. Troubleshooting

Problem	Possible solutions
No or low yield of target fragment after PCR	➢ Increase lysis at 75 °C to 15 – 30 min to improve release of DNA. ➢ If sample reduces pH of lysis reaction to <7.5, increase final concentration of KAPA Express Extract Buffer to 1.5 – 2X. ➢ Dilute DNA extract with TE or 10 mM Tris-HCl, pH 8.0 – 8.5 prior to PCR. ➢ Use at least 40 PCR cycles. ➢ Increase extension time to 30 or 45 sec per cycle. ➢ Decrease annealing temperature by 2 – 5 °C, but not lower than 50 °C.
Smeary or non-specific amplification	➢ Reduce number of PCR cycles to 35 or less. ➢ Reduce annealing time to 15 sec and extension time to 5 – 15 sec per cycle. ➢ Increase annealing temperature by 2 – 5 °C. ➢ Prepare fresh primer stocks.

For detailed information on the extraction and amplification of DNA from samples such as FFPE tissue, fish tissues (for barcoding applications) or blood samples, please refer to the appropriate KAPA Express Extract Application Notes, which are available at www.kapabiosystems.com.

6. Storage, handling and specifications

6.1 Shipping, storage and handling

KAPA Express Extract Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, store the entire kit at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, all kit components will retain full activity for at least six months from the date of receipt, or until the expiry date indicated on the kit.

Always ensure that the 10X KAPA Express Extract Buffer is fully thawed and has been vortexed before use.

KAPA Express Extract Buffer and enzyme may be stored at 4 °C for regular, short-term use (up to 1 week). Provided that it has been handled carefully and not contaminated, the kit components are not expected to be compromised if left (unintentionally) at room temperature for short periods of time (up to 24 h). Long-term storage at room temperature or 4 °C is not recommended. Please note that reagents stored above -20 °C are more prone to degradation when contaminated by the user; storage at such temperatures is therefore at the user's own risk.

6.2 Quality control

Each batch of KAPA Express Extract Buffer and enzyme is subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

6.3 Product use limitations and licenses

KAPA Express Extract Kits are developed, designed and sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual component, has been 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.

For technical support, please contact **support@kapabiosystems.com**

Boston, Massachusetts, United States
600 West Cummings Park, Suite 5350
Woburn, MA 01801 U.S.A.
Tel: +1 781 497 2933 Fax: +1 781 497 2934

Cape Town, South Africa
2nd Floor, Old Warehouse Building, Black River Park,
Fir Road, Observatory 7925, Cape Town, South Africa
Tel: +27 21 448 8200 Fax: +27 21 448 6503

Email: info@kapabiosystems.com