KAPA Mouse Genotyping Kit

Kit components	
KK7302 500 x 25 µl reactions	KAPA Mouse Genotyping Kits include: - 2 x 500 U KAPA Express Extract (1 U/µl) - 2 x 5 ml KAPA Express Extract Buffer (10X) - 1 x 6.25 ml KAPA2G Fast Genotyping Mix with dye (2X)
KK7352 500 x 25 µl reactions	KAPA Mouse Genotyping Kits (HotStart) include: - 2 x 500 U KAPA Express Extract (1 U/µl) - 2 x 5 ml KAPA Express Extract Buffer (10X) - 1 x 6.25 ml KAPA2G Fast HotStart Genotyping Mix with dye (2X)

1. Product Description

KAPA Mouse Genotyping Kits include KAPA Express Extract, a novel thermostable protease and buffer system that allows the extraction of PCR-ready DNA from mouse tissue in as little as 15 minutes, and KAPA2G Fast Genotyping Mix with dye, containing a DNA polymerase engineered via a process of molecular evolution for high processivity and extreme speed. The combination of KAPA Express Extract and KAPA2G Fast Genotyping Mix allows for the reliable extraction and amplification of DNA fragments from mouse tissue in 1 hour, as compared to ≥1 day with conventional protocols.

The KAPA Express Extract system has been designed for optimal tissue lysis and sample preservation. 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. Unlike existing protocols that rely on proteinase K digestion, extractions using 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 for sample loss and contamination. The process yields sufficient template for multiple assays and is easily scaled to handle samples in a 96-well format. Each extraction yields a sufficient amount of template for up to 100 x 25 µl PCRs, and may be diluted in TE Buffer for long-term storage at -20 °C.

DNA extracted with KAPA Express Extract is then amplified with KAPA2G Fast Genotyping Mix (2X) with dye. The master mix is designed for routine Fast PCR, offering significant reductions in total reaction times (20 – 70%), as well as improved performance, compared to wild-type Taq DNA polymerase. Reduction in cycle time can be achieved without the requirement for specialized PCR consumables or thermocyclers. KAPA2G Fast Genotyping Mix (2X) is a ready-to-use master mix containing all components for Fast PCR, except primers and template. The 2X master mix contains KAPA2G Fast DNA Polymerase (with or without HotStart modification), KAPA2G Fast PCR Buffer, dNTPs (0.2 mM each dNTP at 1X), $MgCl_2$ (1.5 mM at 1X) and stabilizers. The master mix with dye additionally contains two inert dyes, which allow for the analysis of reaction products by gel electrophoresis directly after completion of the PCR, i.e. without the need to add a DNA loading solution.

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 mouse tissue in a simple, single-tube protocol (15 min), without the need for hazardous chemicals or multiple washing steps.
- Use 1 µl DNA extract per 25 µl PCR. One extraction yields sufficient template for up to 100 x 25 µl PCRs if undiluted lysate is used.
- DNA extracts may be diluted in TE Buffer (1:10) for long-term storage at -20 °C.
- KAPA2G Fast Genotyping Mixes contain a novel DNA Polymerase (with or without HotStart), engineered specifically for Fast PCR, dNTPs (0.2 mM each dNTP final) and $MgCl_2$ (1.5 mM final).
- Use annealing time of 15 sec or less.
- Use 10 sec total extension time for most amplicons <1 kb. Extension time can be increased to 30 sec/kb for longer or more difficult amplicons.
- Do not exceed 25 µl PCR reaction volumes.
- KAPA2G Fast Genotyping Mix with dye allows for direct loading of PCR products into agarose gels, without the addition of a DNA loading dye solution.

DNA fragments generated with KAPA2G Fast Genotyping Mix have the same characteristics as DNA fragments generated with wild-type Taq polymerase and may be used for routine downstream analyses or applications, including restriction enzyme digestion, cloning and sequencing. Like wild-type Taq, KAPA2G Fast DNA Polymerase has 5′-3′ polymerase and 5′-3′ exonuclease activities, but no 3′-5′ exonuclease (proofreading) activity. The fidelity of KAPA2G Fast is similar to that of wild-type Taq; it has an error rate of approximately 1 error per 1.7 x 10^5 nucleotides incorporated. PCR products generated with KAPA2G Fast Genotyping Mix are 3′-dA-tailed and may be cloned into TA cloning vectors. In the HotStart formulation, the enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This eliminates spurious amplification products resulting from non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

2. Applications

KAPA Mouse Genotyping Kits are ideally suited for the rapid extraction and amplification of DNA from mouse tail and ear tissue. Kits are also suitable for the extraction and amplification of DNA from other animal tissue but protocol optimization may be required.

3. DNA extraction protocol

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

Step	Description	
1. Reaction setup	Combine the following in a thin-walled PCR tube and vortex to mix:	
	PCR-grade water	Up to 100 µl
	1 U/µl KAPA Express Extract Enzyme	2.0 µl
	10X KAPA Express Extract Buffer	10 µl
	Mouse tail or ear tissue	~2 mm^3 fragment or 2 mm^2 punch
2. Lysis[1]	Incubate in a thermocycler for 10 min at 75 °C. During this step, cells are lysed, nucleases and proteins degraded and DNA released[2].	
3. Heat-inactivation[1]	Incubate for 5 min at 95 °C to inactivate the thermostable KAPA Express Extract protease.	
4. Sample recovery and use	1. Vortex reaction product for 2 – 3 sec. Centrifuge at high speed for 1 min to pellet debris[3]. 2. Transfer DNA-containing supernatant (~70 µl) to a fresh tube[4]. 3. Use 1 µl of DNA extract directly in a 25 µl PCR, without quantification. 4. Dilute in TE Buffer for long-term storage at -20 °C (optional).	

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

[2]The tissue will not degrade completely, as is the case with Proteinase K digestions. This is normal, and not an indication of lysis failure.

[3]The centrifugation step is optional. If the lysate will be stored long-term, then centrifugation and transfer to a fresh tube prior to dilution in TE is recommended.

[4]Transferring the lysate to a fresh tube is only required if the centrifugation will be performed.

Notes:

- **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.
- **Lysis and heat-inactivation:** The lysis step at 75 °C may be varied between 5 and 15 min, but shorter or longer lysis times are not recommended. Inadequate lysis will result in low DNA yields, whereas excessive incubation at 75 °C may result in DNA damage. Please note that after the lysis reaction the tissue will not be completely degraded, as is the case with Proteinase K digestions. This is normal, and not an indication of lysis failure.
- **Recovery of DNA-containing extract after lysis and enzyme de-activation (optional):** Centrifugation of the reaction product for 1 min at high speed is sufficient to pellet cellular debris, and the supernatant is easily recovered (~70 µl). 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.
- **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 may be stored at -20 °C for use in multiple PCRs over a period of time. A 1:10 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.

4. DNA amplification protocol

4.1 Typical reaction setup:

A typical reaction with KAPA2G Fast Genotyping Mix consists of the following:

Component	Final concentration	Volume in a 25 µl[1] rxn
PCR grade water	–	Up to 25.0 µl
2X KAPA2G Fast Genotyping Mix	1X	12.5 µl
$MgCl_2$ (25 mM) **ONLY if final concentration >1.5 mM needed**	1.5 mM in 1X Master Mix	0.5 µl for each 0.5 mM $MgCl_2$ >1.5 mM
Forward primer (10 µM)	0.50 µM	1.25 µl
Reverse primer (10 µM)	0.50 µM	1.25 µl
DMSO (for amplicons with a GC content >60%)	5.0 – 7.5%	1.25 - 1.875 µl of a 100% solution
Template DNA	-	1 µl extract from KAPA Express Extract lysis reaction

[1]For reaction volumes smaller than 25 µl, scale the volumes of all components down proportionately. Reaction volumes >25 µl are not recommended.

4.2 Recommended KAPA2G Fast Genotyping Mix cycling parameters

Cycling step	Parameters
Initial denaturation	3 min at 95 °C
Denaturation	15 sec at 95 °C
Annealing	15 sec at optimal Ta (55 – 65 °C)[1]
Extension	10 - 30 sec/kb[2] at 72 °C
No. of cycles	35[3]
Final extension[4]	0 – 10 min at 72 °C

[1]For optimal results, design primers to have an optimal annealing temperature between 55 and 65 °C. Primers with lower annealing temperatures may be used, but annealing temperatures <45 °C are not recommended. Never exceed an annealing time of 15 sec per cycle, as this may lead to non-specific amplification and/or smearing.

[2]10 sec/kb is recommended for most amplicons less than 1< kb. The extension time can be increased up to 30 sec/kb for amplicons that amplify with low yield.

[3]The optimal number of cycles depends on template concentration. Start with 35 and increase or reduce as needed.

[4]A final extension is only required if 3'-dA-tailing is required for fragment analysis or cloning into TA cloning vectors.

5. Troubleshooting

Problem	Possible solutions
No or low yield of target fragment after PCR	➢ Increase lysis at 75 °C to 15 min to improve release of DNA. ➢ 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 20 or 30 sec per cycle. ➢ Decrease annealing temperature by 2 – 5 °C, but not lower than 50 °C. ➢ Ensure tail/ear tissue is completely immersed in buffer.
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 – 10 sec per cycle. ➢ Increase annealing temperature by 2 – 5 °C. ➢ Prepare fresh primer stocks.

6. Storage, handling and specifications

6.1 Shipping, storage and handling

KAPA Mouse Genotyping 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 all kit components are fully thawed and vortexed before use.

KAPA Mouse Genotyping Kits 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

All components contained with KAPA Mouse Genotpying 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.

6.3 Product use limitations and licenses

KAPA Mouse Genotyping 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.

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