KAPABIOSYSTEMS

Technical Data Sheet

KAPA Mouse Genotyping Kit

KR0385_S – v1.17

Product Description

KAPA Mouse Genotyping Kits are designed for rapid extraction and amplification of DNA from mouse tissue. Kits include KAPA Express Extract, a novel thermostable protease and buffer system that enables the extraction of PCR-ready DNA from tissues in as little as 15 minutes. It also includes KAPA2G Fast Genotyping Mix with dye (with or without HotStart), which contains a DNA polymerase engineered through a process of directed 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 contains a thermostable protease, designed for rapid sample lysis. Lysis is performed in a thermocycler, heating block or waterbath, after which the sample is centrifuged to recover the DNA-containing supernatant. The supernatant is then diluted 10-fold prior to use as template in PCR. 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, or multiple wash steps. This greatly reduces the risk of sample loss and contamination. The process yields sufficient template for multiple assays and is easily scaled to handle samples in 96-well format. Each extraction yields a sufficient amount of template for at least 100 genotyping PCR reactions. Extracts (either diluted or undiluted) are stable at -20°C for at least 6 months.

DNA extracted with KAPA Express Extract is amplified with KAPA2G Fast Genotyping Mix with dye. KAPA2G Fast Genotyping Mix (2X) is a ready-to-use master mix containing all components for fast PCR, except primers and template. The 2X ReadyMix contains KAPA2G Fast DNA Polymerase (with or without antibody-mediated HotStart), KAPA2G Buffer A, dNTPs (0.2 mM each at 1X), $MgCl_2$ (1.5 mM at 1X) and stabilizers. The ReadyMix also contains two inert dyes, allowing for the analysis of PCR reaction products by agarose gel electrophoresis without the need to add a DNA loading solution.

DNA fragments generated with KAPA2G Fast Genotyping Mix are 3'-dA-tailed and may be cloned into TA cloning vectors, or used for routine downstream analyses or applications, including restriction enzyme digestion, cloning and sequencing. Like wild-type *Taq*, KAPA2G Fast 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.

Kit Codes and Components	
KK7301 (100 reactions) **KK7302** (500 reactions)	1 U/µL KAPA Express Extract Enzyme 10X KAPA Express Extract Buffer 2X KAPA2G Fast Genotyping Mix with dye
KK7351 (100 reactions) **KK7352** (500 reactions)	1 U/µL KAPA Express Extract Enzyme 10X KAPA Express Extract Buffer 2X KAPA2G Fast HotStart Genotyping Mix with dye
KK5121 (6.25 mL)	**PCR Mix only:** 2X KAPA2G Fast Genotyping Mix with dye
KK5620 (1.25 mL) **KK5621** (6.25 mL)	**PCR Mix only:** 2X KAPA2G Fast HotStart Genotyping Mix with dye
KK7103 (500 extractions)	**Extraction only:** 1 U/µL KAPA Express Extract Enzyme 10X KAPA Express Extract Buffer

Quick Notes

- Extract PCR-ready DNA from mouse tail, ear, or toe tissue in 15 minutes.
- Dilute DNA extract 10-fold prior to PCR in 10 mM Tris-HCl (pH 8.0–8.5), and use 1 µL of DNA per 25 µL reaction.
- Extracts are stable at -20°C for >6 months.
- KAPA2G Fast (HotStart) Genotyping Mix contains the engineered KAPA2G Fast DNA Polymerase for fast PCR, along with buffer, $MgCl_2$ (1.5 mM at 1X), and dNTPs (0.2 mM each at 1X).
- Loading dye for electrophoresis is included in the KAPA2G Fast Genotyping Mix, which allows direct loading of PCR products onto agarose gels for analysis.
- Use 15 sec annealing time, with 15 sec extension time for amplicons <1 kb, or 15–30 sec/kb extension for longer amplicons.

Product Applications

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

For more information about the KAPA Mouse Genotyping Kits, refer to the **Mouse Genotyping Application Note**, available from **www.sigmaaldrich.com**.

KAPA Express Extract Protocol

Step 1: Prepare the PCR master mix

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	N/A
10X KAPA Express Extract Buffer	10 µL	1X
1 U/µL KAPA Express Extract Enzyme	2 µL	2 U/rxn
Mouse tissue	As required	2 mm section

[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 of tissue per 100 µL reaction.

Step 2: Lysis

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

Step	Temperature	Duration
Lysis	75°C	10 min
Enzyme inactivation	95°C	5 min

NOTE: Unlike digestions 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.

Step 3: Dilution

Centrifuge samples briefly to pellet cellular debris (optional), and dilute the DNA extract 10-fold with 10 mM Tris-HCl (pH 8.0–8.5). Extracts are stable (either diluted or undiluted) at 4°C for at least 1 month, and at -20 °C for at least 6 months.

Alternative DNA extraction methods
KAPA2G Fast Genotyping Mixes can be used with DNA extracted from mouse tissues using a variety of methods, including extractions with commercial DNA isolation kits, Proteinase K digestion, and alkaline lysis. We recommend determining the optimal amount of DNA extract for use in genotyping PCR by performing PCR with a serial dilution of template DNA. Please contact Technical Support at **sigma-aldrich.com/techservice** for assistance.

KAPA2G Fast Genotyping PCR Protocol

IMPORTANT! KAPA2G Fast Genotyping Mixes contain an engineered DNA polymerase and uniquely-formulated buffer, and requires specialized reaction conditions. If these conditions are not adhered to, reaction failure is likely.

Step 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 reaction[1]	Final conc.
PCR-grade water	Up to 25 µL	N/A
2X KAPA2G Fast (HotStart) Genotyping Mix with dye[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	As required[3]	10 ng or 1 µL of crude extract

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

[2] KAPA2G Fast Genotyping Mixes contain 1.5 mM $MgCl_2$ at 1X. Additional $MgCl_2$ may be added if necessary.

[3] Use 10 ng of purified genomic DNA, or 1 µL of DNA extracted with crude extraction methods. For DNA extracted with KAPA Express Extract, use 1 µL of the 1:10 dilution (prepared in Step 3 of **KAPA Express Extract Protocol**) as template.

- 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.

Step 2: PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95ºC	3 min	1
Denaturation	95ºC	15 sec	35–40
Annealing	60ºC	15 sec	
Extension	72ºC	15 sec/kb	
Final extension	72ºC	1 min/kb	1

Important Parameters

DNA extraction

KAPA Express Extract is capable of rapid and highly efficient digestion of mammalian tissues. Unlike Proteinase K, KAPA Express Extract Enzyme does not degrade the tissue completely. Visible tissue remaining in the tube after the extraction is not an indication of reaction failure.

The lysis step may be adjusted between 5–15 min, but longer or shorter times are not recommended. Shorter lysis times may limit the yield of DNA, while longer lysis times can result in DNA damage.

The recommended 10-fold dilution of the DNA extracted with KAPA Express Extract dilutes the DNA and any inhibitory compounds to levels that will not negatively impact the downstream PCR, and also ensures long-term stability of the extract. Using the undiluted extract may result in poor PCR performance.

Amplicon size and GC content

Mouse genotyping assays typically target amplicons <1 kb. For amplicons above 2 kb in size, the use of crude extraction methods is not recommended; these assays should be attempted using purified genomic DNA instead. The KAPA2G Fast Genotyping Mixes are capable of amplifying fragments up to 5 kb in size from purified mouse genomic DNA.

For GC-rich amplicons (>65% GC content), reactions may be supplemented with 5% DMSO. If results are poor, the KAPA2G Robust HotStart ReadyMix may be used, which is optimized for GC-rich PCR.

Multiplex assays

Multiplex assays with **up to** three primer pairs can be performed with the KAPA Mouse Genotyping Kit, provided that the annealing temperature and relative primer concentrations have been optimized. Multiplex PCR is likely to require higher $MgCl_2$ concentrations than singleplex PCR. For highly-multiplexed assays, we recommend the KAPA2G Fast Multiplex PCR Kit, with purified mouse genomic DNA as template.

Primers

Primers should be designed to eliminate the possibility of primer-dimer formation and nonspecific annealing, and should have a GC content of 40–60%. Primers with a GC content >60% may require higher denaturation temperatures and/or longer denaturation times, while primers with a GC content <40% may require annealing temperatures <60 °C, and/or increased $MgCl_2$ and primer concentrations. Furthermore, primer sets should be designed to have similar theoretical melting temperatures.

NOTE: Always dilute and store primers in a buffered solution (e.g. 10 mM Tris-HCl, pH 8.0–8.5) instead of PCR-grade water to limit degradation and maintain primer quality.

Cycling protocol

Due to the use of the engineered KAPA2G Fast DNA Polymerase in the KAPA2G Fast Genotyping Mixes, it is critical that the correct cycling conditions are used. The use of protocols typically required by wild-type *Taq* (with 1 min extension times) is highly likely to result in reaction failure with the KAPA Mouse Genotyping Kit. Follow the protocol provided closely to ensure successful PCR.

The unique buffering system of the KAPA2G Fast Genotyping Mix is designed to facilitate primer annealing across a wide range of primer and amplicon lengths and GC contents. This means that for most assays, an annealing temperature of 60°C can be used with high success rates. However, if 60°C does not produce the desired result, adjustments should be made as follows:

- If a low yield of only the specific product is obtained, lower the annealing temperature in 2°C increments.
- If nonspecific products are formed in addition to the specific product, increase the annealing temperature in 2°C increments.
- If no product is formed (specific or nonspecific), reduce the annealing temperature by 5°C. $MgCl_2$ concentration may have to be increased.

Annealing temperature gradient PCR is a quick and easy method to determine the optimal annealing temperature for a mouse genotyping assay. Refer to the **Mouse Genotyping Application Note** for more information.

Product Specifications

Shipping, storage and handling

KAPA Mouse Genotyping 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 Fast Genotyping Mixes 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. Reagents may be stored at 4°C for short-term use (up to 1 month). Return to -20°C for long-term storage. Provided that all components have been handled carefully and not contaminated, the kit is not expected to be compromised if left (unintentionally) at room temperature for a short period of time (up to 3 days).

Quality control

All components contained with KAPA Mouse Genotyping 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.

Troubleshooting

Symptoms	Possible causes	Solutions
No amplification or low yield	Template DNA concentration	Increase lysis time to 15 min to improve release of DNA. Increase the amount of template DNA per reaction to a maximum of 50 ng, or 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.
	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 to 30 sec/kb. 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.
	$MgCl_2$	Optimize $MgCl_2$ concentration. AT-rich and Multiplex PCR typically require more $MgCl_2$.
Nonspecific amplification or smearing	Template DNA	Reduce either the amount of template DNA, or the number of cycles.
	Cycling protocol	Excessive annealing and/or extension times will result in nonspecific amplification, typically of bands larger than the target band. Reduce the annealing and extension times to a minimum of 10 sec each.
	Annealing temperature is too low	A sub-optimal annealing temperature will result in nonspecific amplicons that are typically smaller than the target band. See **Important Parameters: Cycling protocol**.
	High target GC content	Supplement reactions with 5% DMSO, or try the KAPA2G Robust HotStart ReadyMix.
	Primer concentration	Some primers anneal more efficiently than others. Decrease the primer concentration. Store and dilute primers in a buffered solution, not water.

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