

 **KAPA2G™ Fast Multiplex PCR Kit**

Kit codes and components

KK5801 100 reactions	KAPA2G Fast Multiplex Mix (2X) 1 x 1.25 ml (100 x 25 µl rxns)
KK5802 500 reactions	KAPA2G Fast Multiplex Mix (2X) 1 x 6.25 ml (500 x 25 µl rxns)

KAPA2G Fast Multiplex PCR Kits contain MgCl₂ at a 1X concentration of 3.0 mM.

Storage, handling and specifications

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

Quick Notes

- KAPA2G Fast Multiplex PCR Kits contain all the components for Fast Multiplex PCR except primers and template.
- The novel KAPA2G Fast HotStart DNA Polymerase (supplied at 1 U/25 µl rxn) offers faster cycling times and improved reaction efficiency for the even amplification of all target fragments.
- The Multiplex Mix contains 0.2 mM of each dNTP and 3.0 mM MgCl₂ at 1X final concentration.
- Use 0.2 µM of each primer, and 10 – 100 ng of template DNA.
- Anneal for 30 sec at 60 °C.
- Extend for 15 sec at 72 °C. Increasing the extension time up to 90 sec/cycle may be required for longer amplicons or highly multiplexed reactions.
- Do not perform more than 30 cycles.

1. Product Description

KAPA2G Fast Multiplex PCR Kits are designed for Fast Multiplex PCR, offering faster cycling times and improved reaction efficiency compared to conventional Multiplex PCR assays performed with wild-type Taq DNA polymerase.

KAPA2G Fast Multiplex Mix (2X) is a ready-to-use cocktail containing all components for Fast Multiplex PCR, except primers and template. The Multiplex Mix contains KAPA2G Fast HotStart DNA Polymerase (1 U per 25 µl reaction), KAPA2G Buffer A (1.5X at 1X), dNTPs (0.2 mM each dNTP at 1X), MgCl₂ (3.0 mM at 1X) and stabilizers.

KAPA2G Fast HotStart DNA Polymerase is an antibody-mediated hot start formulation of KAPA2G Fast DNA Polymerase, a second-generation enzyme engineered via molecular evolution. KAPA2G Fast DNA Polymerase was optimized for higher processivity and speed, offering significantly faster extension rates than wild-type Taq DNA polymerase. In the HotStart formulation, the enzyme is combined with a proprietary antibody which inactivates the enzyme until the first denaturation step. This eliminates spurious amplification products resulting from non-specific primer events during reaction setup and initiation, and improves overall reaction efficiency.

Multiplex PCR is a challenging application that typically requires more optimization than standard, single amplicon PCR assays. The key to successful Multiplex PCR is the ability to define a single set of reaction parameters (reagent concentrations and cycling parameters) that allows for all primers to anneal with high specificity to their target sequences and be extended with the same efficiency. Primer design, as well as the enzyme and buffer system, are critical factors in this challenge.

The KAPA2G Fast Multiplex PCR Kit offers faster and more efficient Multiplex PCR than competitor enzymes based on wild-type Taq DNA polymerase. The increased speed and processivity of the engineered KAPA2G Fast HotStart DNA Polymerase results in a significant decrease in cycling times, whilst still maintaining the ability to efficiently amplify difficult fragments. The uniquely formulated KAPA2G Fast Multiplex Mix facilitates primer annealing and highly specific amplification of a wide range of amplicon sizes and GC contents, resulting in more even amplification of all target fragments.

2. Applications

The KAPA2G Fast Multiplex PCR Kit is ideally suited for end-point, Fast Multiplex PCR of multiple DNA fragments, ranging in size from 50 – 1,500 bp. Up to 30 different primer pairs may be combined into a single assay using the protocols provided in this Technical Data Sheet.

The kit is ideally suited for:

- Typing of transgenic organisms
- Amplification of microsatellites
- Typing and detection of pathogens
- Amplification of multiple DNA fragments for SNP genotyping

3. Reaction setup

A typical Multiplex PCR with the KAPA2G Fast Multiplex PCR Kit consists of the following:

	Final concentration	Volume in a 25 µl ¹ rxn
PCR grade water	–	Up to 25.0 µl
KAPA2G Fast Multiplex Mix (2X)	1X	12.5 µl
Forward primer (10 µM)	0.20 µM	0.50 µl
Reverse primer (10 µM)	0.20 µM	0.50 µl
Template DNA	As needed	10 – 250 ng

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

4. Cycling parameters

The recommended cycling protocols for Fast Multiplex PCR with the KAPA2G Fast Multiplex PCR Kit are outlined in the table below:

CYCLING STEP	LOW PLEX	MEDIUM PLEX	HIGH PLEX
	≤5 amplicons, ≤1000 bp ≤10 amplicons, ≤500 bp	≤10 amplicons, ≤1000 bp ≤20 amplicons, ≤500 bp	≤10 amplicons, ≤1500 bp ≤30 amplicons, ≤1000 bp
Initial denaturation	3 min at 95 °C	3 min at 95 °C	3 min at 95 °C
Denaturation	15 sec at 95 °C	15 sec at 95 °C	15 sec at 95 °C
Annealing ¹	30 sec at 60 °C	30 sec at 60 °C	30 sec at 60 °C
Extension ²	15 – 30 sec at 72 °C	30 – 60 sec at 72 °C	60 – 90 sec at 72 °C
No. of cycles ³	30	30	30
Final extension (optional) ⁴	1 – 10 min at 72 °C	1 – 10 min at 72 °C	1 – 10 min at 72 °C

¹Annealing should be performed for 30 sec/cycle. Start with an annealing temperature of 60 °C.

²Minimum extension time is 15 sec/cycle. Depending on the size and complexity of the Multiplex assay, up to 90 sec/cycle can be used (particularly for long DNA fragments, or highly multiplexed reactions).

³Do not perform more than 30 cycles, as this results in uneven amplification of fragments.

⁴Final extension is only required if fragments are to be TA-cloned, or if reaction products will be analyzed by fluorescent capillary electrophoresis.



5. Optimization

The conditions in Sections 3 and 4 represent a general approach to Multiplex PCR which has been shown to produce even yields of all amplicons with a number of Multiplex Assays, ranging in complexity from 2-plex to 20-plex, and in amplicon size from 100 bp – 1 kb.

If the conditions in Sections 3 and 4 do not yield satisfactory results, the following parameters may be optimized:

5.1 Annealing time and temperature

Annealing time should be at least 30 sec. Some assays may benefit from annealing times of up to 1 min. The recommended annealing temperature is 60 °C. For primers with a high melting temperature, an annealing temperature of up to 68 °C may be used. Annealing temperatures below 60 °C are not recommended, as they may result in non-specific amplification.

5.2 Template DNA

Multiplex PCR assays typically require higher amounts of template than standard PCR assays. Start with at least 10 ng of genomic DNA per reaction, and at least 1 ng of less complex DNA (e.g. plasmid, lambda).

5.3 Extension time

The recommendations in Section 4 serve as a guideline for determining the optimal extension time. If longer fragments are not amplified well (or at all), the extension time may be increased by 15 – 30 sec/cycle.

5.4 Primer concentration

The recommended primer concentration is 0.2 μM of each primer. However, some primer sets amplify less efficiently than others, and would benefit from increased primer concentration. For example, if the yield of an amplicon is low compared to others in the assay, increasing the primer concentration for that amplicon may be helpful to increase its yield. Primer concentrations of up to 1 μM have been used successfully, but do not use excessive primer concentrations unnecessarily, as this may promote the formation of primer-dimer and other non-specific reaction products.

5.5 MgCl₂ concentration

The KAPA2G Fast Multiplex Mix contains 3 mM MgCl₂ (at 1X), which is optimal for most assays. However, additional MgCl₂ may benefit highly complex assays, as well as assays with amplicons above 1 kb. Any PCR grade MgCl₂ solution may be used.

5.6 Amplicon GC content

For best results, all the amplicons in a Multiplex assay should have similar GC contents. Typically, Multiplex PCR works best with amplicon GC contents of 35 – 55%. Amplicons with GC contents ≥60% should not be combined with lower GC amplicons. For Multiplex PCR of GC-rich amplicons (60 – 70% GC), we recommend adding either 5% DMSO, or 1 M betaine to reactions. GC-rich amplicons typically require longer extension times. Start with at least 30 sec extension per cycle.

5.7 Amplicon length

For best results, the amplicons in a Multiplex assay should not fall outside of a 1 kb range of amplicon sizes. This may result in preferential amplification of the shorter amplicons, which would result in an uneven yield (or failure to amplify the longer amplicons). Should experimental design dictate a difference in product size >1 kb, the primer concentration of the longer amplicon may have to be increased to compensate for differences in amplification efficiency.

5.8 Primer design and storage

Before attempting Multiplex PCR, all primer pairs should first be validated in single reactions to confirm specificity. The yield of a specific primer pair in a single reaction gives some indication of how efficiently it is amplified – this information can be used to empirically decide on relative primer concentrations in the event that uneven yields are achieved in a Multiplex PCR assay (see Section 4). Primer quality is critical for Multiplex PCR. Primers should be dissolved and stored in 10 mM Tris-HCl, pH 8 – 8.5, and never in water. Do not subject primers to multiple freeze-thaw cycles – rather keep small aliquots, or store primers at 4 °C.

6. Specifications

6.1 Shipping, storage and handling

KAPA2G Fast Multiplex PCR Kits are shipped on ice packs. Upon receipt, store the entire kit at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, the KAPA2G Fast Multiplex PCR Kit will retain full activity until the expiry date indicated on the kit.

The KAPA2G Fast Multiplex Mix (2X) contains isostabilizers and may not freeze solidly, even when stored at -20 °C. Nevertheless, always ensure that the Multiplex Mix is fully thawed and has been vortexed before use. The Multiplex Mix is stable for up to 50 freeze-thaw cycles, but repeated freezing and thawing should be avoided.

KAPA2G Fast Multiplex PCR Kits may be stored at 4 °C for regular, short-term use (up to 1 month). Provided that the kit has been handled carefully and not contaminated, the Multiplex Mix is not expected to be compromised if left (unintentionally) at room temperature for short periods of time (up to 3 days). 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

KAPA2G Fast DNA Polymerase and its proprietary HotStart antibody are extensively purified through the use of multiple chromatography steps. The final formulation contains <2% contaminating protein, as determined in an Agilent Protein 230 Assay. Each batch of enzyme, buffer and other components 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

KAPA2G Fast Multiplex PCR Kits are developed, designed and sold exclusively for research purposes and in vitro use. Neither the product, nor any individual component, was 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.

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