KAPABIOSYSTEMS

Technical Data Sheet

KAPA LongRange HotStart ReadyMix PCR Kit

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Product Description

KAPA LongRange HotStart ReadyMix with dye (2X) is a ready-to-use cocktail containing all components for short- to long-range PCR, except primers and template. The 2X ReadyMix contains KAPA LongRange HotStart DNA Polymerase (0.675 U per 25 µL reaction), KAPA LongRange Buffer (1X), dNTPs (0.3 mM of each dNTP at 1X), $MgCl_2$ (2 mM at 1X) and stabilizers. The 2X ReadyMix also contains two inert tracking dyes to enable direct loading of PCR products onto agarose gels for analysis by electrophoresis, without the need to add a DNA loading solution.

KAPA LongRange HotStart DNA Polymerase is a blend of KAPA Taq HotStart DNA polymerase (with antibody hotstart to inactivate the enzyme until the first denaturation step, prevent nonspecific amplification during reaction setup, increase sensitivity and improve reaction efficiency) and a modified archaeal (Type B) DNA polymerase possessing proofreading capability. This two-enzyme system is designed to support robust, long range, and sensitive PCR. Both enzymes possess 5'→3' polymerase activity, but only KAPA Taq HotStart DNA polymerase possesses 5'→3' exonuclease activity and only the Type B DNA polymerase possesses 3'→5' exonuclease (proofreading) activity. The fidelity of KAPA LongRange HotStart is 2–4 fold higher than that of *Taq* DNA Polymerase, but is lower than the fidelity of pure proofreading polymerases such as KAPA HiFi. Reaction products generated with KAPA LongRange HotStart ReadyMix are polyA-tailed, and can be cloned into TA-cloning vectors.

KAPA LongRange HotStart ReadyMix is designed to perform robust, long-range and/or sensitive PCR and should be used when *Taq* DNA polymerase cannot support a PCR because the target is too long or the template DNA concentration is too low. The blend can also be used to replace *Taq* DNA polymerase in standard reactions, but is particularly suitable in cases where the yield of the PCR is low due to the performance limitations of *Taq* DNA polymerase. When KAPA LongRange HotStart ReadyMix is used in reactions that work well with *Taq* DNA Polymerase, there is no improvement in yield, but there is an improvement in fidelity.

Product Applications

KAPA LongRange HotStart ReadyMix is ideally suited for:

- PCR of amplicons to be analyzed by agarose gel electrophoresis
- PCR of short and medium length targets (<5 kb)
- Long-range PCR (5 – 15 kb)
- PCR with limiting amounts of template DNA.

Kit Codes and Components	
KK3601 (1.25 mL)	2X KAPA LongRange HotStart ReadyMix with dye
KK3602 (6.25 mL)	(contains 2 mM $MgCl_2$ at 1X)

Quick Notes

- KAPA LongRange HotStart is a blend of KAPA Taq HotStart and a proofreading DNA polymerase.
- Suitable for short-, medium- and long-range PCR, and PCR with low amounts of template DNA.
- Fidelity is 2–4 times better than *Taq*.
- For short amplicons, replace *Taq* DNA polymerase for improvements in yield and/or fidelity.
- Amplify mid- to long-range targets with high yield and sensitivity.
- 2X ReadyMix with dye contains two inert tracking dyes to allow loading of PCR products directly onto agarose gels for analysis.

Product Specifications

Shipping and Storage

KAPA LongRange HotStart ReadyMix PCR kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -15°C to -25°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. The 2X KAPA LongRange HotStart ReadyMix contains isostabilizers and may not freeze solidly, even when stored at -15°C to -25°C. This will not affect the shelf-life of the product.

Handling

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 2°C to 8°C for short-term use (up to 1 month). Return to -15°C to -25°C for long-term storage.

Quality Control

Each batch of KAPA LongRange HotStart DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). The 2X KAPA LongRange HotStart ReadyMix is subjected to stringent quality control tests, is free of contaminating exo- and endonuclease activity, and meets strict requirements with respect to DNA contamination levels.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.
- Safety Data Sheets (SDS) are available online at www.sigmaaldrich.com, or upon request from www.sigma-aldrich.com/techservice

KAPA LongRange HotStart PCR Protocol

KAPA LongRange HotStart ReadyMix can be used to replace any commercial *Taq* DNA polymerase in an existing protocol to improve yield and/or fidelity. It can also be used to amplify mid- to long-range targets (up to 15 kb). Template DNA quality is critical to ensure successful long-range amplification.

Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- 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 volumes of each component based on the following table:

Component	25 µL reaction[1]	Final conc.
PCR-grade water	Up to 25 µL	N/A
2X KAPA LongRange HotStart ReadyMix with dye (2 mM $MgCl_2$ at 1X)[2]	12.5 µL	1X
10 µM Forward Primer	0.5–1.25 µL	0.2–0.5 µM
10 µM Reverse Primer	0.5–1.25 µL	0.2–0.5 µM
Template DNA[3]	As required	As required

[1] Reaction volumes of 10–50 µL are recommended. For volumes other than 25 µL, scale reagents proportionally.

[2] A final $MgCl_2$ concentration of 2 mM is sufficient for most standard applications. For assays that do not perform well with 2 mM $MgCl_2$, the optimal $MgCl_2$ concentration for each primer/template combination should be determined empirically.

[3] ≤250 ng for genomic DNA; ≤25 ng for less complex DNA (e.g. plasmid, lambda).

NOTE: For GC-rich or other difficult templates or amplicons, include DMSO at a final concentration of 5%.

Step 2: Set up individual reactions

NOTE: Always invert and/or pipette mix input material for long-range PCR so as not to damage the DNA. Avoid vortexing where possible before addition to the reaction mix.

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

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	3 min[1]	1
Denaturation[2]	95°C	30 sec	35[5]
Annealing[3]	T_m – 5°C	30 sec	
Extension[4]	72°C	1 min/kb	
Final extension (optional)[6]	72°C	1 min/kb	1
Hold	4–10°C	∞	1

[1] Initial denaturation for 3 min at 95°C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95°C may be used.

[2] For long targets, cycle denaturation can be done at 94°C for 15 sec (slow-ramping cyclers) or 25 sec (for fast-ramping cyclers).

[3] An annealing temperature 5°C lower than the calculated melting temperature (T_m) of the primer pair is recommended as a first approach. If low yields and/or non-specific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

[4] For long-range targets, extend at 68°C.

[5] 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring higher sensitivity, while lower cycle numbers can be used if the template copy number is high. If high fidelity is required, keep the number of cycles as low as possible.

[6] Final extension should be included if PCR products are to be cloned into TA cloning vectors.

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