KAPABIOSYSTEMS

Technical Data Sheet

KAPA Taq EXtra PCR Kit

KR0365_S – v1.17

Product Description

The KAPA Taq EXtra PCR system is a blend of KAPA Taq DNA polymerase and a modified archaeal (Type B) DNA polymerase possessing proofreading capability. This two-enzyme system is designed specifically to support robust, long range, and sensitive PCR. Both enzymes possess 5'→3' polymerase activity, but only KAPA Taq DNA polymerase possesses 5'→3' exonuclease activity and only the Type B DNA polymerase possesses 3'→5' exonuclease (proofreading) activity. The fidelity of KAPA Taq EXtra is 2–4 fold higher than that of KAPA Taq DNA Polymerase, but is lower than the fidelity of pure proofreading polymerases, such as KAPA HiFi. Reaction products generated with KAPA Taq EXtra are polyA-tailed, and can be cloned into TA-cloning vectors.

The KAPA Taq EXtra system 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 wild-type *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 Taq EXtra DNA Polymerase is used as a replacement for *Taq* in reactions that are easily supported by *Taq*, there is no improvement in yield, but there is an improvement in fidelity.

Product Applications

The KAPA Taq EXtra PCR system is ideally suited for:

- PCR of short- and medium-length targets (<5 kb)
- Long-range PCR (5–15 kb)
- PCR with limiting amounts of template DNA.

Product Specifications

Shipping and Storage

KAPA Taq EXtra PCR kits are shipped on dry ice or ice packs, depending on the country of destination. 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. KAPA Taq EXtra Buffer contains isostabilizers and may not freeze solidly, even when stored at -20°C. This will not affect the shelf-life of the product.

Kit Codes and Components	
KK3009 (250 U)	KAPA Taq EXtra DNA Polymerase (5 U/μL) KAPA Taq EXtra Buffer (5X) $MgCl_2$ (25 mM) KAPA dNTP Mix (10 mM each)

Quick Notes

- KAPA Taq EXtra is a blend of KAPA Taq 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.
- KAPA Taq EXtra Buffer (Mg-free) is designed for long-range, sensitive PCR. $MgCl_2$ is supplied separately for optimization.

Handling

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.

Quality Control

Each batch of KAPA Taq EXtra DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA Taq EXtra PCR kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

KAPA Taq EXtra PCR Protocol

KAPA Taq EXtra 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 and dNTP quality are critical to ensure successful long-range amplification.

Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed, and that you work on ice throughout 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 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
5X KAPA Taq EXtra Buffer	5.0 µL	1X
25 mM $MgCl_2$	1.5 µL	≥1.5 mM[2]
10 mM dNTP Mix	0.5 µL	0.2 mM each
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
5 U/µL KAPA Taq EXtra DNA Polymerase[3]	0.125 µL	0.675 U
Template DNA[4]	As required	As required[5]

[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 1.5 mM is sufficient for most standard applications. For assays that do not perform well with 1.5 mM $MgCl_2$, the optimal $MgCl_2$ concentration for each primer/template combination should be determined empirically.

[3] For GC-rich and other difficult templates, higher enzyme concentrations (up to 2.5 U per 25 µL reaction) may be required.

[4] ≤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	20–40[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 content), 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 set is recommended as first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature for the primer set empirically.

[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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