

1. Product Description

KAPATaq HotStart DNA Polymerase is based on the single-subunit, wild-type *Taq* DNA polymerase of the thermophilic bacterium *Thermus aquaticus*. In the HotStart formulation, the enzyme (which is purified from recombinant *E. coli*) 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 and sensitivity. Antibody-based hot start PCR enzymes offer superior performance compared to hot start formulations based on chemical modification of the enzyme, as enzyme re-activation is faster, more complete and is optimal at pH values which are also optimal for polymerase activity.

KAPATaq HotStart DNA Polymerase has 5'-3' polymerase and 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. The enzyme has an error rate of approximately 1 error per 2.2×10^5 nucleotides incorporated. PCR products generated with KAPATaq HotStart are A-tailed and may be cloned into TA cloning vectors.

KAPATaq HotStart Buffer is a novel buffer designed for optimal enzyme re-activation and performance. The proprietary formulation also facilitates specific primer annealing, which translates to higher yields of specific product when compared to traditional *Taq* buffers. The 5x buffer is supplied without $MgCl_2$ for optimal flexibility. KAPATaq HotStart DNA Polymerase may, however, be used in combination with any standard *Taq* buffer with a pH of 8.3 or higher.

2. Applications

KAPATaq HotStart DNA Polymerase is ideally suited for the following applications:

- High-throughput PCR.
- Amplification of low copy DNA targets.
- Multiplex PCR.
- Specific amplification of complex targets.
- RT-PCR.

Kit components*	Product codes		
	KK 1514	KK 1508 1509	KK 1510 1511
KAPATaq HotStart DNA Polymerase (5 U/ μ l)	100 U	250 U	500 U
5x KAPATaq HotStart Buffer without $MgCl_2$	1.5 ml	3.0 ml	6.0 ml
$MgCl_2$ (25 mM)	1.6 ml	1.6 ml	1.6 ml
dNTP mix (10 mM each)	-	300 μ l (KK1509 only)	600 μ l (KK1511 only)

*For the larger kits, please refer to our website

Storage

Store all components at $-20^\circ C$.

Quick Notes

- Antibody-based HotStart formulation and novel buffer offers superior specificity, sensitivity and yields.
- Enzyme re-activation is complete in 30 sec. However, longer initial denaturation times are needed for complex genomic and GC-rich templates.
- Use 0.5 units KAPATaq HotStart DNA Polymerase per 25 μ l reaction for standard applications.
- Use 1 min/kb extension time.



3. Reaction setup

A typical KAPATaq HotStart reaction consists of the following⁵:

Component	Final concentration	Volume in a 25 µl reaction ¹
PCR grade water		Up to 25.0 µl
5x KAPATaq HotStart Buffer ²	1x	5.0 µl
MgCl ₂ (25 mM) ³	1.5 - 5.0 mM	0.5 µl for each 0.5 mM MgCl ₂
dNTP mix (10 mM each)	0.2 mM each dNTP	0.50 µl
Forward primer (10 µM)	0.1 - 1.0 µM	0.25 µl for each 0.1 µM needed (e.g. 1.25 µl for 0.5 µM final)
Reverse primer (10 µM)	0.1 - 1.0 µM	0.25 µl for each 0.1 µM needed (e.g. 1.25 µl for 0.5 µM final)
Template DNA	As needed	≤250 ng for genomic DNA ≤25 ng for less complex DNA (e.g. plasmid, lambda)
KAPATaq HotStart DNA Polymerase ⁴ (5 units/µl)	0.5 - 2.5 units/25 µl rxn	0.10 µl for each 0.5 U needed

Notes on reaction setup:

1. Reaction volumes of 10 - 50 µl are recommended. For volumes larger or smaller than 25 µl, scale reagents listed in the above table up or down proportionally.
2. Ensure that all components are fully thawed before use. Vortex the 5x KAPATaq HotStart Buffer before each use.
3. A final MgCl₂ concentration of 1.5 mM is sufficient for most standard applications. The optimal MgCl₂ concentration for each primer-template combination should be determined empirically in a MgCl₂ gradient PCR.
4. 0.5 units KAPATaq HotStart DNA Polymerase per 25 µl reaction should be sufficient for most assays. For GC-rich or other difficult templates, higher enzyme concentrations are likely to improve results. The enzyme has been validated with up to 2.5 units per 25 µl reaction.
5. For GC rich or other difficult templates or amplicons, include DMSO to a final concentration of 5 - 6 % in the reaction.

For advanced troubleshooting or assistance with reaction setup or optimization, consult the KAPATaq HotStart FAQs and other web-based technical resources on <http://www.kapabiosystems.com> or e-mail support@kapabiosystems.com.



4. Cycling parameters

A typical KAPATaq HotStart cycling profile is outlined below.

Step	Temp (°C)	Time	No. of cycles
Initial denaturation ¹	95°C	30 sec for low complexity templates 3 min for genomic or GC-rich DNA	1
Denaturation	95°C	10 - 30 sec	25 - 45 (See Note 5)
Primer annealing ^{2,6}	45 - 68°C	10 - 30 sec	
Extension ³	72°C	1 min/kb	
Final extension (OPTIONAL) ⁴	72°C	30 - 60 sec/kb	1
Cooling	4 - 10°C	HOLD	1

Notes on cycling parameters:

1. KAPATaq HotStart enzyme is fully re-activated within 30 sec, but longer initial denaturation times are required to fully denature complex or GC-rich templates. For recalcitrant templates, the initial denaturation may be increased to a maximum of 5 min.
2. For primers with an optimal annealing temperature (Ta) between 68 and 72°C, a 2-step protocol with a combined annealing/extension step of 60 - 90 sec/kb at 68 - 72°C may be used.
3. For AT rich templates and amplicons, extension may be performed at 68°C.
4. A final extension is only necessary if PCR products are to be cloned into TA-cloning vectors.
5. The number of cycles depends on the amount of starting material (target copy number) in the reaction. The following may be used as a general guideline:

>10 ⁶ copies	25 cycles
10 ⁴ - 10 ⁶ copies	30 cycles
<10 ⁴ copies	35+ cycles

The approximate target copy number may be calculated using the formula:

$$(M \times 1,515) / bp \times (6.022 \times 10^{11}) \times P$$

where M = mass in µg of template DNA in the reaction, bp = number of base pairs of total template (not target) DNA and P = number of priming sites of primer pair on template

e.g. the target copy number for a single copy gene in 1 ng human genomic DNA equals: $(1 \times 10^{-3}) \times 1,515 / (3.3 \times 10^9) \times (6.022 \times 10^{11}) \times 1 \approx 280$ copies

6. When designing primers, the theoretical melting temperature (Tm) of primers used together should be matched as closely as possible. As a first approach, use an annealing temperature (Ta) 3- 5°C lower than the lowest Tm of the two primers. For best performance, the optimal Ta for a primer pair should be determined empirically by Ta gradient PCR. Because primer melting characteristics are affected by the chemical environment, the optimal Ta for a specific primer pair should be determined in the PCR buffer used for the assay and may differ from one buffer system to another. In some cases, sample composition may also affect primer annealing.

5. Specifications

5.1 Shipping and storage

KAPATaq HotStart 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 one year from the date of receipt.

5.2 Handling

Always ensure that all kit components are fully thawed before use. Vortex 5x KAPATaq HotStart Buffer after each freeze-thaw cycle. Return components to -20°C for long-term storage.

5.3 Quality control

KAPATaq 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 are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

5.4 Product use limitations and licenses

KAPATaq HotStart 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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For technical support please contact support@kapabiosystems.com