

## KAPA Taq PCR Kit

KR0352 – v6.16

### Product Description

KAPA Taq DNA Polymerase is the single-subunit *Taq* DNA polymerase of the thermophilic bacterium *Thermus aquaticus*, purified from recombinant *Escherichia coli*. KAPA Taq DNA Polymerase has 5'→3' polymerase and 5'→3' exonuclease activity, but no 3'→5' exonuclease (proofreading) activity. The enzyme system has an error rate of approximately 1 error per 2.2 x 10<sup>5</sup> nucleotides incorporated. PCR products generated with KAPA Taq are A-tailed and are suitable for cloning into TA cloning vectors.

KAPA Taq DNA Polymerase is supplied with two reaction buffers (Buffer A and Buffer B) or a single buffer with loading dye, allowing convenient direct analysis of PCR product by agarose gel electrophoresis after cycling. KAPA Taq Buffer A (and KAPA Taq Buffer with dye) are standard Tris-ammonium sulphate-based buffers, while KAPA Taq Buffer B is a Tris-potassium chloride buffer. All KAPA Taq Buffers are 10X buffers, containing 15 mM MgCl<sub>2</sub> (1.5 mM at 1X). KAPA Taq DNA Polymerase may, however, be used in combination with any standard *Taq* buffer with a pH of 8.3 or higher.

### Product Applications

KAPA Taq DNA Polymerase is ideally suited for:

- Routine PCR
- Amplification of DNA for Sanger sequencing
- Any standard PCR application for which a high-quality thermostable DNA polymerase is required.

### Product Specifications

#### Shipping and Storage

KAPA Taq 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.

#### 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 DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA Taq 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/Roche Kit Codes and Components		
<b>KK1014</b> 07958579001	250 U	
<b>KK1015</b> 07958587001	500 U	KAPA Taq DNA Polymerase (5 U/μL) KAPA Taq Buffer A (10X)
<b>BK1000</b> 07958447001	2500 U	KAPA Taq Buffer B (10X) MgCl <sub>2</sub> (25 mM)
<b>BK1002</b> 07958463001	5000 U	
<b>KK1008</b> 07958552001	250 U	
<b>KK1016</b> 07958595001	500 U	KAPA Taq DNA Polymerase (5 U/μL) KAPA Taq Buffer A (10X) KAPA Taq Buffer B (10X)
<b>BK1001</b> 07958455001	2500 U	MgCl <sub>2</sub> (25 mM) KAPA dNTP Mix (10 mM each)
<b>BK1003</b> 07958471001	5000 U	
<b>KK1020</b> 07958617001	250 U	
<b>KK1022</b> 07958633001	500 U	KAPA Taq DNA Polymerase (5 U/μL) KAPA Taq Buffer w/loading dye (10X)
<b>BK1004</b> 07958498001	2500 U	MgCl <sub>2</sub> (25 mM)
<b>BK1006</b> 07958510001	5000 U	
<b>KK1021</b> 07958625001	250 U	
<b>KK1023</b> 07958641001	500 U	KAPA Taq DNA Polymerase (5 U/μL) KAPA Taq Buffer w/loading dye (10X)
<b>BK1005</b> 07958501001	2500 U	MgCl <sub>2</sub> (25 mM) KAPA dNTP Mix (10 mM each)
<b>BK1007</b> 07958528001	5000 U	

Quick Notes
<ul style="list-style-type: none"> <li>• KAPA Taq DNA Polymerase can replace any commercial <i>Taq</i> DNA polymerase in an existing protocol. The final MgCl<sub>2</sub> concentration may need to be optimized to account for differences in buffer formulation.</li> <li>• KAPA Taq Buffers contain MgCl<sub>2</sub> at a final concentration of 1.5 mM.</li> <li>• Buffer A is recommended as first approach and for applications requiring high yields.</li> <li>• Buffer B is recommended for applications where high sensitivity is required (e.g. when the template is limiting).</li> <li>• Both buffers may be evaluated to determine the buffer most suitable for a specific application.</li> <li>• The KAPA Taq PCR system is suitable for the amplification of fragments up to 3.5 kb from genomic DNA or 5 kb from less complex targets.</li> </ul>

## KAPA Taq PCR Protocol

KAPA Taq DNA Polymerase can be used to replace any commercial *Taq* DNA polymerase in an existing protocol. To allow the most seamless integration of KAPA Taq into existing protocols, be sure to match reaction conditions, particularly the  $MgCl_2$ , primer and enzyme concentrations, as closely as possible.

### 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 $\mu$ L reaction <sup>1</sup>	Final conc.
PCR-grade water	Up to 25 $\mu$ L	N/A
10X KAPA Taq Buffer <sup>2</sup>	2.5 $\mu$ L	1X
25 mM $MgCl_2$	As required <sup>3</sup>	$\geq 1.5$ mM
10 mM dNTP Mix	0.5 $\mu$ L	0.2 mM each
10 $\mu$ M Forward Primer	1.0 $\mu$ L	0.4 $\mu$ M
10 $\mu$ M Reverse Primer	1.0 $\mu$ L	0.4 $\mu$ M
5 U/ $\mu$ L KAPA Taq DNA Polymerase <sup>4</sup>	0.1 $\mu$ L	0.5 U
Template DNA	As required	As required <sup>5</sup>

<sup>1</sup> Reaction volumes of 10–50  $\mu$ L are recommended. For volumes other than 25  $\mu$ L, scale reagents proportionally.

<sup>2</sup> KAPA Taq Buffer A, KAPA Taq Buffer B and KAPA Taq Buffer with dye all contain a final  $MgCl_2$  concentration of 1.5 mM at 1X.

<sup>3</sup> For assays requiring  $>1.5$  mM  $MgCl_2$ , the reaction may be supplemented with additional  $MgCl_2$  as required.

<sup>4</sup> For GC-rich and other difficult templates, higher enzyme concentrations (up to 2.5 U per 25  $\mu$ L reaction) may be required.

<sup>5</sup>  $\leq 250$  ng for genomic DNA;  $\leq 25$  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%.



**Headquarters, United States**  
**Wilmington, Massachusetts**  
 Tel: 781.497.2933  
 Fax: 781.497.2934

**Manufacturing, R&D**  
**Cape Town, South Africa**  
 Tel: +27.21.448.8200  
 Fax: +27.21.448.6503

**Technical Support**  
[kapabiosystems.com/support](http://kapabiosystems.com/support)

**Sales**  
[sales@kapabiosystems.com](mailto:sales@kapabiosystems.com)

### Step 2: Set up individual reactions

- 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 <sup>1</sup>	1
Denaturation	95°C	30 sec	35 <sup>3</sup>
Annealing <sup>2</sup>	$T_m - 5^\circ C$	30 sec	
Extension	72°C	1 min/kb	
Final extension (optional) <sup>4</sup>	72°C	1 min/kb	1
Hold	4–10°C	$\infty$	1

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring a higher level of sensitivity.

<sup>4</sup> Final extension should be included if PCR products are to be cloned into TA cloning vectors.