



## KAPA HiFi™ HotStart DNA Polymerase

### Product code

KK2501

KK2502

### Kit size

100 units

250 units

## 1. Product Description

KAPAHiFi HotStart DNA Polymerase is a novel, single-enzyme system that exhibits industry-leading performance when compared with other high fidelity polymerases and polymerase blends. KAPAHiFi HotStart is recommended for amplifying targets up to 18 kb for plasmid or lambda and up to 15 kb for genomic DNA.

In the HotStart formulation, the enzyme 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.

KAPAHiFi HotStart DNA Polymerase kits include two buffers for optimal performance with difficult templates.

## 2. Applications

KAPAHiFi HotStart is ideally suited for high fidelity PCR where amplified product is cloned for use in downstream applications such as:

- site-directed mutagenesis
- sequencing
- protein expression

## 3. Background

Processivity is defined as the number of nucleotides incorporated by the polymerase per binding event, and is a major determinant of extension speed and robustness. In contrast to fusion technologies that attach DNA binding proteins to Pfu-like polymerases to improve processivity, KAPAHiFi HotStart DNA Polymerase has been engineered to have an increased affinity for DNA without the need for accessory protein domains. The intrinsic high processivity of KAPAHiFi HotStart results in significant improvements in yield, sensitivity, speed, target length and the ability to amplify difficult templates.

When performing high-fidelity PCR, it is important to minimize the number of cycles required to yield sufficient product for cloning. Additional cycles increase yield at the expense of fidelity: the small number of errors introduced early in the reaction are fixed in the population, and are amplified with each successive cycle. During subsequent cycles, any additional errors will successively increase the proportion of mutant amplicons in the population.

Please note that both the cycling temperature and time parameters for KAPAHiFi HotStart will be different to those required for non-engineered high fidelity polymerases such as Pfu or Vent.

### Kit Components

- KAPAHiFi HotStart DNA Polymerase (1 U/μl in storage buffer)
- 5x KAPAHiFi Fidelity Buffer with MgCl<sub>2</sub>\*
- 5x KAPAHiFi GC Buffer with MgCl<sub>2</sub>\*
- 25 mM MgCl<sub>2</sub>
- KAPA dNTP Mix (10 mM each dNTP)

\*Both buffers contain Mg<sup>2+</sup> at a 1x concentration of 2.0 mM.

### Storage

Store all components at -20°C.

### Quick Notes

- Denature at 98°C for 20 seconds.
- Use 30 sec/kb; longer extension times may improve yield and sensitivity.
- Use KAPAHiFi HotStart in GC Buffer for difficult templates.
- KAPAHiFi HotStart produces blunt end DNA products.

Enzyme	KAPAHiFi HotStart	Pfu	Taq
Origin	Engineered	<i>Pyrococcus furiosus</i>	<i>Thermus aquaticus</i>
Error rate* (errors per nt)	2.8 x 10 <sup>-7</sup>	2.2 x 10 <sup>-6</sup>	2.6 x 10 <sup>-5</sup>
Initial elongation rate (nt/sec)	50 - 75	25	61
Processivity (nt)**	>100	>20	>42

\*Fidelity is determined by direct DNA sequencing of PCR amplicons generated with KAPAHiFi™ HotStart on a Roche GS FLX sequencer. Pfu and Taq are published error rates.

\*\*Processivity is defined as the number of nucleotides that can be extended in one catalytic reaction by a single molecule of DNA polymerase.



## 4. Reaction setup

The standard reactions conditions outlined below should provide satisfactory results in most cases. For specific primer/template combinations or applications, further optimization may be achieved by varying the concentrations of enzyme (0.25 - 1.0 U per 25 µl reaction), Mg<sup>2+</sup>, template and/or primers. KAPAHiFi Buffers contain Mg<sup>2+</sup> at a 1x concentration of 2.0 mM. Additional Mg<sup>2+</sup> may be required for some primer/template combinations. The optimal Mg<sup>2+</sup> for each specific application may be determined in a Mg<sup>2+</sup> gradient PCR, where the final Mg<sup>2+</sup> concentration is increased in increments of 0.25 mM.

Inclusion of a positive and a negative (no template) control reaction in each experiment is recommended. For the positive control, use template DNA known to yield a positive result with the specific primer pair.

- For each 25 µl reaction, assemble the following:

PCR grade water	Up to 25.0 µl
5x KAPAHiFi Fidelity Buffer* or 5x KAPAHiFi GC Buffer (final concentration 1x)	5.00 µl
dNTP mix (10 mM each dNTP; final concentration 0.3 mM)	0.75 µl
Forward primer (10 µM; final concentration 0.3 µM)	0.75 µl
Reverse primer (10 µM; final concentration 0.3 µM)	0.75 µl
Template DNA (≤50 ng genomic DNA, ≤5 ng plasmid or lambda)	As needed
KAPAHiFi HotStart DNA Polymerase (1 U/µl)	0.50 µl
<b>Final volume</b>	<b>25.0 µl</b>

\*Fidelity Buffer is recommended for most assays. GC Buffer is recommended for difficult templates with high GC content or assays where Fidelity Buffer produces low yield.

- Mix and centrifuge briefly to collect reaction components to the bottom of the tube. Begin thermal cycling immediately.

## 5. Cycling parameters

Initial Denaturation: 95°C 2 - 5 min

Denaturation:	98°C	20 sec
Annealing:	T <sub>m</sub> +/- 10°C	15 sec
Extension:	72°C	30 sec/kb

15 - 35 cycles

Final Extension: 72°C 1 - 5 min

### Notes

- The higher concentration of salt in KAPAHiFi reaction buffers affects DNA melting. Complex templates require longer initial denaturation times (up to 5 mins).
- KAPAHiFi HotStart requires a denaturation for 20 seconds at 98°C in each cycle.
- The optimal annealing temperature for each assay should be determined empirically in an annealing temperature gradient PCR (see Section 6.5).
- 30 sec/kb extension time is recommended.
- 25 or fewer cycles are recommended for most high fidelity applications. In cases where very low template concentrations or inefficient amplification results in low yields, 30 or 35 cycles may be performed.
- Successful amplification in the case of longer targets (≥10 kb), low target copy number and/or specific primer/template combinations may be dependant on MgCl<sub>2</sub> concentration. To determine the optimal MgCl<sub>2</sub> for a specific assay, a MgCl<sub>2</sub> gradient PCR, in which the final MgCl<sub>2</sub> concentration is increased in increments of 0.25 mM, is recommended.
- It may be useful to vary enzyme concentration between 0.25 U and 1.0 U per 25 µl reaction.



## 6. Additional Guidelines

### 6.1 KAPAHiFi Buffers

Two 5x buffers are supplied with KAPAHiFi HotStart PCR Kits, namely KAPAHiFi Fidelity Buffer and KAPAHiFi GC Buffer. The Fidelity Buffer is recommended for most reactions. The GC Buffer is specifically formulated for amplicons/templates with high GC content and/or stable secondary structure, and is recommended when the Fidelity Buffer produces a low yield. Both buffers contain  $Mg^{2+}$  at a 1x concentration of 2.0 mM. There is a two-fold decrease in fidelity when using the GC Buffer.

### 6.2 Amplicon length

KAPAHiFi HotStart is recommended for the amplification of plasmid and lambda targets up to 18 kb and genomic targets up to 15 kb. For the efficient amplification of fragments  $\geq 10$  kb from genomic DNA, higher template concentrations and careful optimization of  $Mg^{2+}$  concentration may be required.

### 6.3 Template DNA

Amplification from low complexity templates such as lambda or plasmid DNA is usually easy and should require little optimization. Applications based on low target copy numbers (e.g. when amplifying single copy genes from genomic templates, or when using cDNA as template) are generally more challenging. For plasmid or phage DNA, 5 ng template per 25  $\mu$ l reaction is adequate, whereas up to 50 ng genomic DNA or cDNA may be required. The quality of the template DNA has a very significant impact on the outcome of the PCR. Degraded, damaged or sheared target DNA is particularly problematic when amplifying targets  $>1$  kb. Dilute and store DNA in TE or 10 mM Tris (pH = 8.0 - 8.5) and minimize freeze-thaw cycles to reduce degradation and maintain quality. High quality template DNA is critical for high fidelity PCR.

### 6.4 Extension time

KAPAHiFi HotStart is capable of amplifying targets up to 18 kb in length at an extension rate of 30 sec/kb. Extension times may be further optimized for particular applications. It may be possible to reduce extension times if sufficient template is present, while longer extension times (up to 1 min/kb) may result in significant gains in sensitivity and/or yield.

### 6.5 Primers and annealing temperature

Primer design is important for successful PCR amplification. Primer GC content should be approximately 40-60%. A GC content  $>60\%$  may require a higher denaturation temperature and/or a longer denaturation time. Primer pairs should exhibit similar melting temperatures ( $T_m$ ). Primers for two-step cycling programs should be designed with a high  $T_m$  value to ensure efficient annealing and extension at a single temperature (in the range of 65 - 72°C).

As a first approach, use an annealing temperature equal to the lowest calculated  $T_m$  for the primer pair. To improve **sensitivity**, **reduce** the annealing temperature in increments of 1°C; to improve **specificity**, **increase** the annealing temperature in 1°C increments.

There are several methods for calculating the predicted  $T_m$  of a primer. The nearest-neighbor method (50 mM monovalent salt) is recommended, as it takes the primer sequence and other variables (such as salt and DNA concentration) into account. The most general method of calculating the  $T_m$  of an oligonucleotide is based on the number of adenine (A), thymidine (T), guanidine (G) or cytosine (C) bases:  $T_m$  (°C) =  $2(nA + nT) + 4(nG + nC)$ . Please note that the actual  $T_m$  of a given primer may be affected by specific reaction conditions, including reaction buffer, DNA concentration, presence of denaturants (e.g. DMSO) and nucleotide modifications (e.g. biotin, fluorescent dyes). It is therefore recommended that the optimal annealing temperature for a specific assay be determined empirically in an annealing temperature gradient PCR.

### 6.6 dNTPs

Successful amplification with proofreading polymerases is highly dependent on the quality of the dNTPs used; the presence of even very small amounts of dUTP has a dramatic impact. Use only the highest quality dNTPs from KAPA Biosystems, supplied with your kit. The recommended dNTP concentration of 0.3 mM is sufficient for all standard applications and has been validated for targets up to 18 kb.

### 6.7 $Mg^{2+}$ concentration

Both KAPAHiFi Buffers contain  $Mg^{2+}$  at a 1x concentration of 2.0 mM. This has been found to be sufficient for most applications. Specific applications, particularly those involving the amplification of fragments  $>10$  kb from complex genomic templates, may require careful optimization of the  $Mg^{2+}$  concentration. A  $Mg^{2+}$  gradient PCR, in which the final  $Mg^{2+}$  concentration is increased in increments of 0.25 mM is recommended in such cases.

### 6.8 TA cloning

DNA fragments generated with KAPAHiFi HotStart are suitable for blunt-end cloning. For TA cloning, dA overhangs may be added to blunt-ended KAPAHiFi HotStart PCR products with KAPATaq DNA Polymerase in a final extension step at 72°C. It is very important to purify the PCR product prior to the A-tailing reaction. If this is not done, the proofreading activity of KAPAHiFi HotStart will degrade the dA overhangs.



## 7. Troubleshooting

Symptom	Possible cause	Solution
No PCR Product	<ul style="list-style-type: none"> <li>Target size too large</li> <li>High GC content and/or DNA secondary structure</li> <li>Low amount of template</li> <li>Mg<sup>2+</sup> concentration too low</li> </ul>	<p>Reduce target size. KAPAHiFi HotStart amplifies up to 15 kb genomic DNA and up to 18 kb plasmid and phage DNA targets.</p> <p>Use KAPAHiFi GC Buffer.</p> <p>For plasmid or phage DNA ≥5 ng of template is preferred. Genomic and cDNA templates should be increased up to 50 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.</p> <p>Optimize Mg<sup>2+</sup> concentration by adding MgCl<sub>2</sub> in 0.25 mM steps. Too much Mg<sup>2+</sup> may result in nonspecific amplification and/or smearing.</p>
Smear instead of distinctive DNA band on agarose gel	<ul style="list-style-type: none"> <li>Low template DNA concentration</li> <li>Annealing temperature too low</li> <li>High GC content and/or DNA secondary structure</li> <li>Template DNA is degraded, nicked, sheared, or otherwise damaged</li> <li>Mg<sup>2+</sup> concentration too high</li> </ul>	<p>For plasmid or phage DNA ≥5 ng of template is preferred. Genomic and cDNA templates should be increased up to 50 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.</p> <p>Increase annealing temperature.</p> <p>Use KAPAHiFi GC Buffer.</p> <p>Ensure template DNA is prepared and stored appropriately. Store DNA in TE or 10 mM Tris (pH 8.0 - 8.5). Minimize freeze-thaw cycles.</p> <p>Note that both buffers supplied with the kit already contain Mg<sup>2+</sup>; additional MgCl<sub>2</sub> is usually not required.</p>
Low yield	<ul style="list-style-type: none"> <li>Low amount of template</li> <li>High GC content and/or DNA secondary structure</li> <li>Mg<sup>2+</sup> concentration too low</li> <li>dUTP contamination</li> <li>Primer concentration too low</li> </ul>	<p>For plasmid or phage DNA ≥5 ng of template is preferred. Genomic and cDNA templates should be increased up to 50 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.</p> <p>Use KAPAHiFi GC Buffer.</p> <p>Optimize Mg<sup>2+</sup> concentration by adding MgCl<sub>2</sub> in 0.25 mM steps. Too much Mg<sup>2+</sup> may result in nonspecific amplification and/or smearing.</p> <p>Use only high quality dNTPs suitable for use with proofreading polymerases.</p> <p>Recommended primer concentration is 0.3 μM final. Higher concentrations of primer may be required for longer templates. Make up fresh primer to ensure high quality.</p>

Product warranty and licensing information can be found at [www.kapabiosystems.com](http://www.kapabiosystems.com)

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