KAPA HiFi™ DNA Polymerase

Product code	Kit size
KK2101	100 units
KK2102	250 units

1. Product Description

KAPAHiFi DNA Polymerase is a novel, single-enzyme system that exhibits industry-leading performance when compared with other high fidelity polymerases and polymerase blends. KAPAHiFi is recommended for amplifying targets up to 18 kb for plasmid or lambda and up to 5 kb for genomic DNA.

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

2. Applications

KAPAHiFi 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

Kit Components

- KAPAHiFI DNA Polymerase (1 U/µl in storage buffer)
- 5x KAPAHiFi Fidelity Buffer with $MgCl_2$
- 5x KAPAHiFi GC Buffer **without** $MgCl_2$*
- 25mM $MgCl_2$
- KAPA dNTP Mix (10 mM each nucleotide)

* Requires the addition of Mg^{2+} at 1.75 mM final concentration

Storage

Store all components at –20 ºC.

Quick Notes

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

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. In contrast to fusion technologies that attach DNA binding proteins to Pfu-like polymerases to improve processivity, KAPAHiFi 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 results in significant improvements in yield, 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 will be different to those required for non-engineered high fidelity polymerases such as Pfu or Vent.

Enzyme	KAPAHiFi	Pfu	Taq
Species	Engineered	*Pyrococcus furiosus*	*Thermus aquaticus*
Error rate* (errors per nt)	$1.0x10^{-6}$	$2.2x10^{-6}$	$2.6x10^{-5}$
Initial elongation rate (nt/sec)	50-75	25	61
Processivity**(nt)	>100	>20	>42

*Fidelity is a measurement of mutation frequency in PCR products using a sensitive blue/white phenotypic assay with a 1.4 kb lacI fragment as template.

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

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4. KAPAHiFi PCR Protocol

In many cases, the standard reactions described below will provide satisfactory amplification. Inclusion of a negative control reaction lacking only template and a positive control reaction using a template known to amplify with the primers is recommended.

Concentrations of enzyme, $MgCl_2$, template and primers can be varied to optimize the reaction. Fidelity Buffer already contains Mg^{2+}. The GC Buffer requires the addition of Mg^{2+} at a 1.75 mM final concentration. Additional Mg may be required for both buffers in 0.25 mM steps, depending on the primer/template combination.

Important note: KAPAHiFi possesses a 3'-5' exonuclease activity.

To prevent degradation of the primers, please observe the following precautions:

- Assemble reactions on ice
- Add the polymerase last
- Begin thermal cycling immediately after adding polymerase to the reaction mix

1. For each 50 µl reaction, assemble the following on ice just prior to use:

Component	Volume
PCR grade water up to 50 µl	x µl
5x KAPAHiFi Reaction Buffer* or 5x KAPAHiFi GC Buffer**	10.00 µl
dNTP mix (10 mM each dNTP; final concentration 0.3 mM)	1.50 µl
5' primer (10 µM; final concentration 0.3 µM)	1.50 µl
3' primer (10 µM; final concentration 0.3 µM)	1.50 µl
Template DNA (100 -200 ng total genomic, 10 ng plasmid or lambda)	x µl
KAPAHiFi DNA Polymerase (1 U/µL)	1.00 µl
Final volume	50 µl

*High 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.

**KAPAHiFi GC Buffer requires the addition of magnesium at 1.75 mM final concentration.

2. Mix, and then centrifuge briefly to bring reaction components to the bottom of the tube and immediately begin thermal cycling.

Cycling Instructions

Step	Temperature	Time	
Initial Denaturation:	95ºC	2 min	
Denaturation:	98ºC	20 sec	15 - 35 cycles
Annealing:	T_m	15 sec	
Extension:	68ºC	30 sec/kb	
Final Extension:	68ºC	1 - 5 min	

Notes on Cycling Conditions

- The higher concentration of salt in KAPAHiFi reaction buffer affects DNA melting.
- As a result, KAPAHiFi requires a denaturation temperature of 98°C for 20 seconds.
- The choice of primers affects the annealing temperature. In general, use an annealing temperature at the calculated T_m (melting temperature) of the primers as a starting point, but remember that PCR with some template/primer combinations may benefit from optimization of annealing temperature.
- We recommend 30 s extension time per 1 kb of target. Note that total extension time less than 20 s is not recommended due to the lag between block temperatures and actual reaction temperatures.
- 25 cycles are recommended for most applications. In cases where very low template concentrations or inefficient amplification results in low yields, 30 or 35 cycles may be performed.

Protocol Notes

- Successful amplification in the case of longer targets (3kb and above), low target number, and/or particular primer/template pairs may be sensitive to $MgCl_2$ concentration.
- It may be useful to test additional $MgCl_2$ in steps of 0.25mM, depending on reaction conditions. Note that higher concentrations of dNTPs may require additional $MgCl_2$. GC Buffer requires the addition of magnesium as 1.75 mM final concentration.
- It may be useful to vary enzyme concentration between 0.5 U and 2 U per 50 µl reaction.

5. Additional Guidelines

5.1 Buffers

Two buffers are supplied with KAPAHiFi kits - Fidelity Buffer, and GC Buffer. Fidelity Buffer is recommended for most reactions. GC Buffer is specially formulated to allow amplification of templates with high GC content and/or secondary structure, and generally results in higher yield and sensitivity. Note that GC buffer requires the addition of magnesium at a 1.75 mM final concentration. There is a two-fold descrease in fidelity when using the GC Buffer.

5.2 Primers

Primer design is important for successful PCR amplification. The G/C content should be approximately 40-60% (a G/C content of greater than 60% may require a higher denaturation temperature 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 proper annealing and extension at the same temperature. In general, use an annealing temperature equal to the lowest Tm of the primer pair as a starting point. To improve sensitivity, reduce annealing temperatures 1 °C at a time; to improve specificity increase annealing temperatures in 1 °C steps.

There are several methods for determining the T_m of a primer. The nearest-neighbor method using 50 mM monovalent salt is recommended for accurate Tm prediction. Unlike other methods, the nearest-neighbor method takes into account the primer sequence and other variables such as salt and DNA concentration. The Tm can also be calculated with the % GC method. The most general method of calculating the Tm 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.). However, the exact T_m of a given primer may be affected by DNA concentration, presence of denaturants (e.g., DMSO), and nucleotide modifications (e.g. biotin, fluorescent dyes, etc...).

Note on dNTPs:

Successful amplification using proof-reading polymerases is very 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.

5.3 Target length

KAPAHiFi is recommended for the amplification of plasmid and lambda targets up to 18kb and genomic targets up to 5kb. As genomic target length increases above 2 kb, the reaction may require higher amounts of starting template DNA and further Mg^{2+} optimization.

5.4 Template DNA

Amplification is generally more difficult when there are few copies of the target DNA such as genomic DNA or cDNA, as compared to plasmid or phage DNA. For plasmid or phage DNA, 10 ng is adequate, whereas genomic and cDNA templates may require up to 100 ng. 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 longer targets (>1 kb).

5.5 Extension time

KAPAHiFi is capable of amplifying targets up to 18 kb in length at speeds of 30s/kb. However, extension times may be further optimized for particular reactions. 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..

5. TA cloning

KAPAHiFi is suitable for blunt ended cloning. If TA cloning is required, it can be performed by adding A overhangs to blunt PCR product using KAPATaq in a final extension step at 72°C. It is very important to purify the PCR product prior to the final A-tailing reaction, otherwise the proofreading activity of any remaining KAPAHiFi enzyme will degrade the A overhangs.

6. Troubleshooting

Symptom	Possible Cause	Solution
No PCR product	Target size too large	Use a smaller target size. KAPAHiFi amplies up to 5 kb genomic DNA and up to 18 kb plasmid and phage DNA targets.
	High GC content and/or DNA secondary structure	Use KAPAHiFi GC Buffer (requires the addition of 1.75 mM Mg^{2+} final).
	Low amount of template	For plasmid or phage DNA ≥10 ng of template is preferred. Genomic and cDNA templates should be increased up to 100 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.
	Mg^{2+} concentration too low	Optimize Mg^{2+} concentration by adding $MgCl_2$ in 0.25 mM steps. Too much Mg^{2+} may result in nonspecific amplification and/or smearing.
Smear instead of distinctive DNA band on agarose gel	Low template DNA concentration	For plasmid or phage DNA ≥10 ng of template is preferred. Genomic and cDNA templates should be increased up to 100 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.
	High GC content and/or DNA secondary structure	Use KAPAHiFi GC Buffer (requires the addition of 1.75 mM Mg^{2+} final).
	Template DNA is degraded, nicked, sheared, or otherwise damaged	Ensure template DNA is prepared and stored appropriately.
	Mg^{2+} concentration too high	Note that both buffers supplied with the kit already contain Mg^{2+}; additional $MgCl_2$ is not usually required.
	Reactions were not set up on ice	The reaction should be set up on ice and KAPAHiFi Polymerase should be added last to the PCR reaction mix to prevent degradation of primers and template.
Low yield	Low amount of template	For plasmid or phage DNA ≥10 ng of template is preferred. Genomic and cDNA templates should be increased up to 100 ng per reaction. If addition of more template is not possible, increase the number of amplification cycles.
	High GC content and/or DNA secondary structure	Use KAPAHiFi GC Buffer (requires the addition of 1.75 mM Mg^{2+} final).
	Mg^{2+} concentration too low	Optimize Mg^{2+} concentration by adding $MgCl_2$ in 0.25 mM steps. Too much Mg^{2+} may result in nonspecific amplification and/or smearing.
	dUTP contamination	Use only high quality dNTPs suitable for use with proofreading polymerases.

Product warranty and licensing information can be found at: **www.kapabiosystems.com**

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