Technical Data Sheet

KAPA™ LongRange DNA Polymerase

Product code	Kit size
KK3006	100 units
KK3005	250 units

Kit Components

- 5 U/µl KAPALongRange Polymerase
- 5x KAPALongRange Buffer
- 25 mM $MgCl_2$ solution
- 250 µl KAPA dNTP Mix (10 mM each)

Storage

Store all components at –20ºC.

Quick Notes

- Denature at 94°C, increase denaturation times for fast-block instruments.
- Extend at 68°C, especially for long range/high sensitivity PCR.
- Replace Taq for Standard PCR, with no protocol change.
- Half concentration of enzyme (1.25 U/50 µl) can be used for midrange targets.
- Buffer is supplied at 5x concentration with separate magnesium for optimization.
- Products can be cloned in T-overhang cloning vectors.

1. Production Description

The KAPALongRange PCR system is a blend of *Taq* DNA polymerase and a modified archaeal (Type B) DNA polymerase possessing proofreading capability. This two-enzyme system is designed specifically to support long range and/or sensitive PCR. The KAPALongRange system polymerizes DNA from a primer annealed to a DNA template in the presence of deoxyribonucleotide triphosphates. Both enzymes possess 5-3' polymerase activity, but only *Taq* possesses double strand dependent 5-3' exonuclease activity and only the Type B polymerase possesses 3-5' exonuclease (proofreading) activity.

KAPALongRange displays higher fidelity than *Taq* polymerase. KAPAHiFi DNA Polymerase is recommended for where higher fidelity is required.

2. Applications

The KAPALongRange PCR system is suited for:

- PCR amplification of long targets and/or PCR using low concentrations of template DNA
- Standard short- and mid-range PCR amplification
- Production of PCR products to be used for ligation into 3'-T-overhang cloning vectors

3. Background

Taq polymerase lacks proofreading activity and is unable to efficiently extend beyond misincorporated bases. Mismatched base pairing generates truncated products that accumulate during PCR and contribute to reaction failure if the target is long and/or the template DNA is supplied in low amounts. In contrast, proofreading high fidelity enzymes are extremely accurate, but do not perform well over longer target distances or with low template concentration because the 3-5' exonuclease (proofreading) activity destroys primers and affects sensitivity.

The addition of a proofreading polymerase to *Taq* allows mismatches incorporated at the 3' end of the growing strand to be repaired. Less primer destruction occurs because the ratio of proofreading activity to polymerase activity in the blend is lower than for a pure proofreading polymerase. The resulting polymerase mix is, therefore, able to support PCR of longer targets over more cycles than either enzyme alone. The fidelity is improved as compared to *Taq*, but is lower than that of pure Type B high fidelity polymerases.

4. Long Range PCR Protocols

The KAPALongRange PCR system is designed to perform long range and/or sensitive PCR. The performance of the kit is heavily dependant upon the nature of the primer pair and the quality of the DNA template. Poorly designed primers or damaged template DNA will detract from the performance, and this is particularly acute if the target is long and/or the template DNA is supplied in low amounts.

KAPALongRange Polymerase should be used when *Taq* 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 *Taq* polymerase in standard reactions, but is particularly suitable in cases where the yield of the PCR is low due the limitations of *Taq* polymerase performance. When KAPALongRange Polymerase is used as a replacement for *Taq* polymerase for reactions that are easily supported by *Taq* there is no improvement in yield, but there is an improvement in fidelity.

For simple PCR (short range/high template concentration), KAPALongRange Polymerase can be used instead of *Taq* without modification of the normal *Taq* protocol. However, as the reaction becomes more difficult (longer range/lower template concentration), the PCR conditions must be adjusted by increasing the number of cycles, decreasing extension temperature, increasing the amount of enzyme and using an auto-extend step during the later cycles of the PCR (applied in that order).

Three typical examples are described below.

NOTES

- The KAPALongRange buffer is supplied as a 5x solution.
- The KAPALongRange buffer is supplied without $MgCl_2$.
- Denaturation times must be increased 10 seconds for fast-block instruments, to compensate for temperature lag of tube contents.
- Concentrations of magnesium, template, dNTPs and primers can be varied to optimize the reaction.
- Mg^{2+} levels must be increased if higher than usual template DNA or dNTP concentrations are used.
- To prevent primer degradation and/or the appearance of non-specific products, assemble reactions on ice, add the enzyme last and begin thermal cycling immediately after adding the polymerase to the reaction mix.

KAPALongRange DNA Polymerase

Standard PCR Protocol for short targets (up to 8 kb) and/or high concentrations of template DNA

Typically, the PCR reaction setup might consist of:

	Final concentration	50 µl rxn
PCR grade water up to 50 µl		As required
5x KAPA LR buffer (without Mg^{2+})	1x	10 µl
$MgCl_2$ (25 mM)	1.75 mM	3.5 µl
dNTPs (10 mM each dNTP)	0.3 mM	1.5 µl
Fwd primer (10 µM)	0.5 µM	2.5 µl
Rev primer (10 µM)	0.5 µM	2.5 µl
Template	As required	As required
KAPA LongRange DNA Polymerase (5 U/µl)	1.25 U / 50 µl	0.25 µl
Total		**50 µl**

PCR cycling conditions might consist of:

Initial Denaturation:	94°C	2 min	
Denaturation:	94°C	15 sec - 25 sec*	25 cycles
Annealing:	T_m -5°C	15 sec	
Extension:	72°C	1 min per kb	
Final Extension:	72 °C	1 min per kb	

*15 sec for slow-block instruments, 25 sec for fast-block instruments.

PCR Protocol for use with longer targets (5 kb to 18 kb) and/or lower concentrations of template DNA

Typically, the PCR reaction setup is similar to 'Standard PCR' and might consist of:

	Final concentration	50 µl rxn
PCR grade water up to 50 µl		As required
5x KAPA LR buffer (without Mg^{2+})	1x	10 µl
$MgCl_2$ (25 mM)	1.75 mM	3.5 µl
dNTPs (10 mM each dNTP)	0.3 mM	1.5 µl
Fwd primer (10 µM)	0.5 µM	2.5 µl
Rev primer (10 µM)	0.5 µM	2.5 µl
Template	As required	As required
KAPA LongRange DNA Polymerase (5 U/µl)	1.25 U / 50 µl	0.25 µl
Total		**50 µl**

Changes to PCR cycling conditions include lowering the extension temperature to 68 °C with the addition of 10 cycles and might consist of:

Initial Denaturation:	94°C	2 min	
Denaturation:	94°C	15 sec - 25 sec*	35 cycles
Annealing:	T_m -5°C	15 sec	
Extension:	68°C	1 min per kb	
Final Extension:	72 °C	1 min per kb	

*15 sec for slow-block instruments, 25 sec for fast-block instruments.

KAPALongRange DNA Polymerase

Long Range PCR protocol for use with very long targets (>15 kb) and/or low concentrations of template DNA

Typically, the PCR mix contains more polymerase and might consist of:

	Final concentration	50 µl rxn
PCR grade water up to 50 µl		As required
5x KAPA LR buffer (without Mg^{2+})	1x	10 µl
$MgCl_2$ (25 mM)	1.75 mM	3.5 µl
dNTPs (10 mM each dNTP)	0.3 mM	1.5 µl
Fwd primer (10 µM)	0.5 µM	2.5 µl
Rev primer (10 µM)	0.5 µM	2.5 µl
Template	As required	As required
KAPA LongRange DNA Polymerase (5 U/µl)	1.25 U / 50 µl	0.25 µl
Total		**50 µl**

Long Range PCR cycling conditions include an auto-extension step and a lower extension temperature and might consist of:

Initial Denaturation:	94°C	2 min	
Denaturation:	94°C	15 sec - 25 sec*	10 cycles
Annealing:	T_m-5°C	15 sec	
Extension:	68°C	1 min per kb	
Denaturation:	94°C	15 sec - 25 sec*	25 cycles
Annealing:	T_m-5°C	15 sec	
Extension:	68°C	1 min per kb + 20 sec per cycle	
Final Extension:	72 °C	1 min per kb	

*15 sec for slow-block instruments, 25 sec for fast-block instruments.

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

For technical support please contact: **support@kapabiosystems.com**

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