Technical Data Sheet

# KAPA™ LongRange HotStart DNA Polymerase

| Product code | Kit size |
|---|---|
| KK3500 | 50 units |
| KK3501 | 100 units |
| KK3502 | 250 units |
| KK3503 | 500 units |

## 1. Product Description

The KAPALongRange HotStart 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. 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.

The KAPALongRange HotStart 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 HotStart displays higher fidelity than *Taq* polymerase. KAPAHiFi™ DNA Polymerase is recommended for applications that require the highest fidelity.

### Kit Components

- 2.5 U/µl KAPALongRange HotStart Polymerase
- 5x KAPALongRange Buffer (without $Mg^{2+}$)
- 25 mM $MgCl_2$ solution
- 250 µl KAPA dNTP Mix (10 mM each)

### Storage

Store all components at –20 °C.

### Quick Notes

- Denature at 94 °C, increase denaturation times for fast-block instruments.
- Extend at 68 °C, especially for long range/high sensitivity PCR.
- For standard PCR, replace Taq 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.

## 2. Applications

The KAPALongRange HotStart 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 blend 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 HotStart 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 HotStart 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 HotStart 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 HotStart 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 magnesium chloride.
- 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.
- Magnesium levels must be increased if higher than usual template DNA or dNTP concentrations are used.

# KAPALongRange HotStart
DNA Polymerase

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

Typically, the PCR reaction setup might consist of:

| | Final concentration | 50µl rxn |
|---|---|---|
| PCR grade water up to 50 µl | | As required |
| 5x KAPALongRange 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 HotStart DNA Polymerase (2.5 U/µl) | 1.25 U / 50 µl | 0.50 µl |
| **Total** | | **50 µl** |

**PCR cycling conditions might consist of:**

| | | | |
|---|---|---|---|
| Initial Denaturation: | 94 °C | 3 min | |
| Denaturation: | 94 °C | 15 sec - 25 sec* | 25 cycles |
| Annealing: | $T_m$ -5 °C | 15 sec | |
| Extension: | 72 °C | 1 min per 1kb | |
| Final Extension: | 72 °C | 1 min per 1kb | |

***15 sec for amplicons up to 5 kb and 25 sec for amplicons > 5 kb.**

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

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

| | Final concentration | 50µl rxn |
|---|---|---|
| PCR grade water up to 50 µl | | As required |
| 5x KAPA LongRange 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 HotStart DNA Polymerase (2.5 U/µl) | 1.25 U / 50 µl | 0.50 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 | 3 min | |
| Denaturation: | 94 °C | 15 sec - 25 sec* | 35 cycles |
| Annealing: | $T_m$ -5 °C | 30 sec | |
| Extension: | 68 °C | 1 min per 1kb | |
| Final Extension: | 72 °C | 1 min per 1kb | |

***15 sec for amplicons up to 5 kb and 25 sec for amplicons > 5 kb.**

# KAPALongRange HotStart DNA Polymerase

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

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

| | Final concentration | 50µl rxn |
|---|---|---|
| PCR grade water up to 50 µl | | As required |
| 5x KAPA LongRange 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 HotStart DNA Polymerase (2.5 U/µl) | 2.5 U / 50 µl | 1.0 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 | 3 min | |
| Denaturation: | 94 °C | 15 sec - 25 sec* | 10 cycles |
| Annealing: | $T_m$ -5 °C | 30 sec | |
| Extension: | 68 °C | 1 min per 1kb | |
| Denaturation: | 94 °C | 15 sec - 25 sec* | 25 cycles |
| Annealing: | $T_m$ -5 °C | 30 sec | |
| Extension: | 68 °C | 1 min per 1kb + 20 sec per cycle | |
| Final Extension: | 72 °C | 1 min per 1kb | |

***15 sec for amplicons up to 5 kb and 25 sec for amplicons > 5 kb.**

### License



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