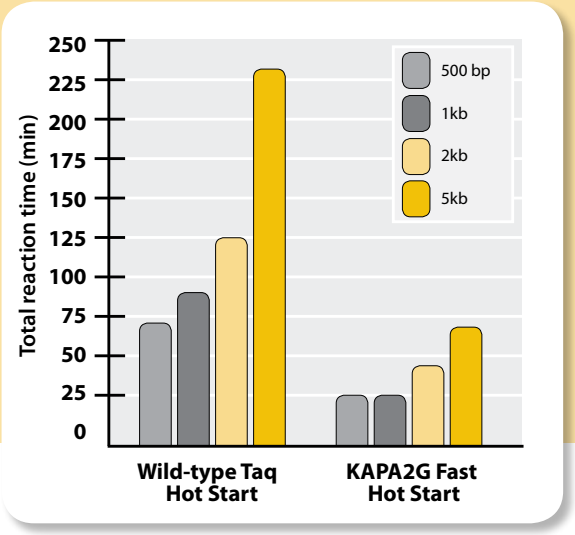
Introducing **KAPA**2G™ Fast HotStart

A second-generation enzyme for the ultimate in speed and performance.

KAPA2G **Fast HotStart combines a second-generation DNA polymerase engineered for extreme speed with HotStart technology for optimal performance.**

The result is industry-leading performance:

- Extension rates as low as 1 sec per kilobase.
- Outstanding performance when compared to conventional hot start Taq polymerase.
- Savings in total reaction time of 20 - 70%.
- No specialist PCR consumables or instrumentation required.



KAPABIOSYSTEMS
www.kapabiosystems.com

next generation thinking in enzyme technology

>> KAPA2G™ Fast HotStart

A second-generation DNA polymerase for the ultimate in speed and performance.

Product Description

KAPA2G Fast HotStart is based on KAPA2G Fast DNA Polymerase, a second-generation enzyme derived through a process of molecular evolution. KAPA2G Fast DNA Polymerase was specifically engineered for higher processivity and speed, offering significantly faster extension rates than wild-type Taq polymerase. 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.

Engineered KAPA2G Fast HotStart and optimized buffer system offers:

- Extension times as low as 1 sec per kilobase.
- Outstanding performance when compared to conventional hot start Taq polymerase.
- 20 - 70% reductions in total PCR time.
- No requirement for specialist PCR consumables or instrumentation.

KAPA2G Fast HotStart is particularly well suited for high throughput Fast PCR, offering total reaction times 20 - 70% shorter than conventional PCR assays performed with wild-type Taq polymerase or hot start formulations thereof. In addition, KAPA2G Fast HotStart has been shown to achieve higher yields and sensitivity than competitor enzymes across a wide range of amplicon types. No specialized PCR consumables or thermocyclers are required.

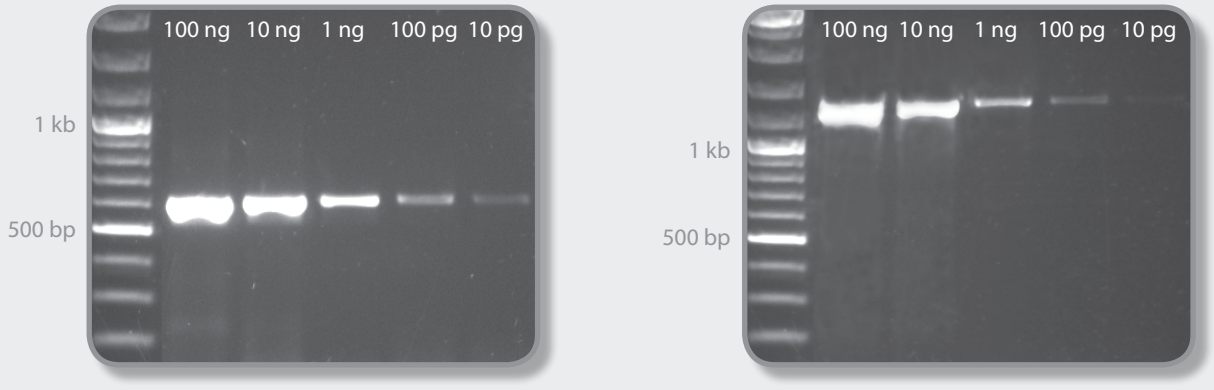
Product Applications

Any standard, end-point PCR assay performed efficiently with wild-type Taq polymerase (or a hot start formulation thereof) may be converted to a Fast PCR assay with KAPA2G Fast HotStart. Amplicons generated with KAPA2G Fast HotStart are suitable for routine downstream applications, including restriction enzyme digestion, cloning and sequencing.

High speed and sensitivity

KAPA2G Fast HotStart is based on a second-generation polymerase with the intrinsic ability to synthesize DNA faster than wild-type Taq and other DNA polymerases. Fast PCR protocols using KAPA2G Fast HotStart are primarily based on reduced extension times that allow for a significant reduction in PCR cycling time without the risk of compromising reaction performance.

Using KAPA2G Fast HotStart, a total extension time of 1 second per cycle is sufficient for the reproducible amplification of fragments <1 kb from as little as a single target copy of human genomic DNA. For amplicons >1 kb, 15 sec/kb extension time per cycle is used.

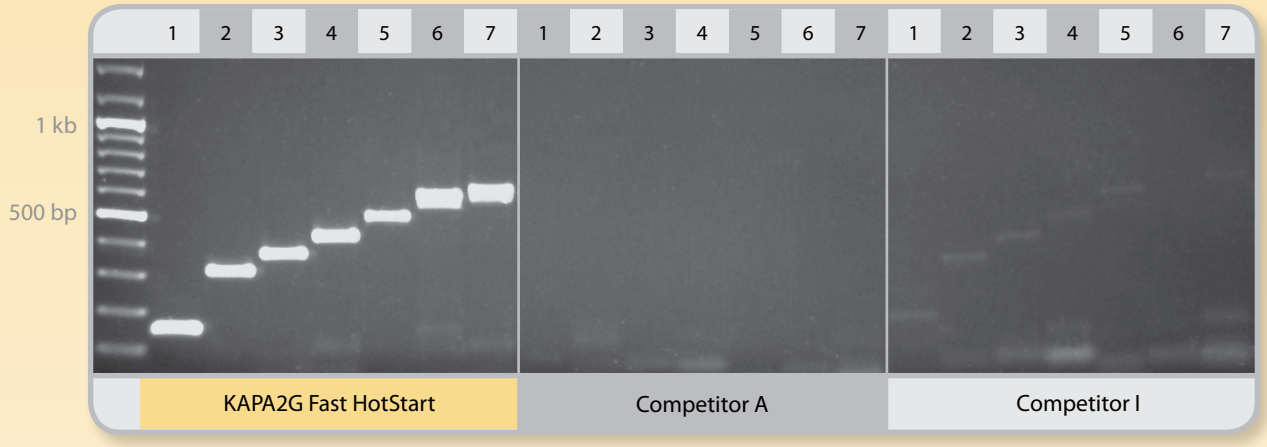


Amplification of a 626 bp fragment of the epidermal growth factor receptor (left) and a 1.3 kb fragment of the macrophage stimulating 1 receptor (right) from a 10-fold dilution series of human genomic DNA using KAPA2G Fast HotStart. 0.5 units of enzyme was used per 25 µl reaction. Cycling was performed on the G-Storm GS1 thermocycler with a fast block, using a standard 3-step cycling profile (35 cycles). For the 626 bp fragment, a total extension time of 1 sec per cycle was used and for the 1.3 kb fragment an extension time of 15 sec/kb (20 sec per cycle).

The ultimate in speed and performance

KAPA2G Fast HotStart and optimized buffer system is capable of dramatically reduced extension times and exceptional performance.

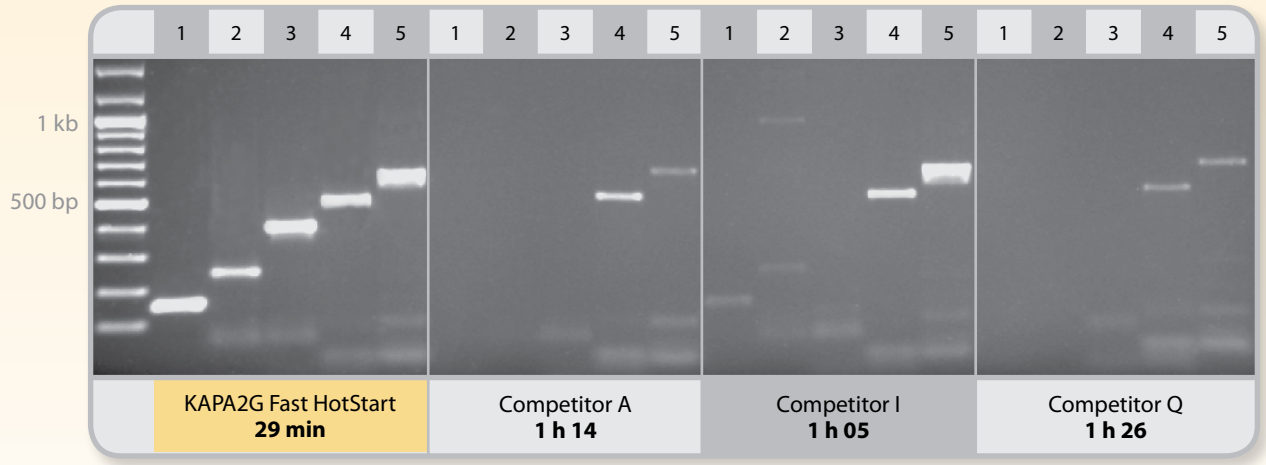
Hot start formulations of wild-type Taq do not perform satisfactorily when cycling times are reduced. In contrast, the unique properties of KAPA2G Fast HotStart allows for efficient amplification in total reaction times that are typically reduced by 40 - 70%.



Fast amplification of 7 human gene fragments using KAPA2G Fast HotStart or competitor hot start Taq formulations. Reactions (25 µl) contained 5 ng human genomic DNA and 0.5 units (KAPA2G Fast HotStart or Competitor I) or 0.625 units (Competitor A) enzyme. Cycling was performed on an Eppendorf Mastercycler ep*gradient* S, using a 3-step (35 cycle) profile with 15 sec denaturation (95ºC), 15 sec annealing (60ºC) and 1 sec extension (72ºC) per cycle. Initial denaturation/ enzyme re-activation time (95ºC) was 2 min for KAPA2G Fast HotStart and Competitor I and 7.5 min for Competitor A. The total reaction time was 29.5 min for KAPA2G Fast HotStart and Competitor I and 37 min for Competitor A.

The gene target, length and GC content of each amplicon are as follows: 1 = 5,10-methylene tetrahydrofolate reductase, 150 bp, 54.0% GC; 2 = zinc finger protein 638, 318 bp, 30.9% GC; 3 = attractin, 373 bp, 37.1% GC; 4 = zinc finger protein 41, 502 bp, 42.5% GC; 5 = PCI domain containing protein 2, 526 bp, 39.3% GC; 6 = chromosome 6 ORF167, 599 bp, 35.6% GC and 7 = epidermal growth factor receptor, 626 bp, 42.0% GC.

KAPA2G Fast HotStart outperforms hot start formulations of wild-type Taq, even when the latter are used at their optimal, slow extension rates (1 min/kb per cycle). The unique properties of this second-generation enzyme, combined with a proprietary HotStart antibody and optimized buffer system allows for efficient fast amplification of a larger variety of amplicon types than competitor enzymes. In the example below, competitor enzymes failed or performed poorly with 40 - 60% of a set of single copy human gene fragments, particularly in cases were amplicons are short or their GC content is high (>65%).



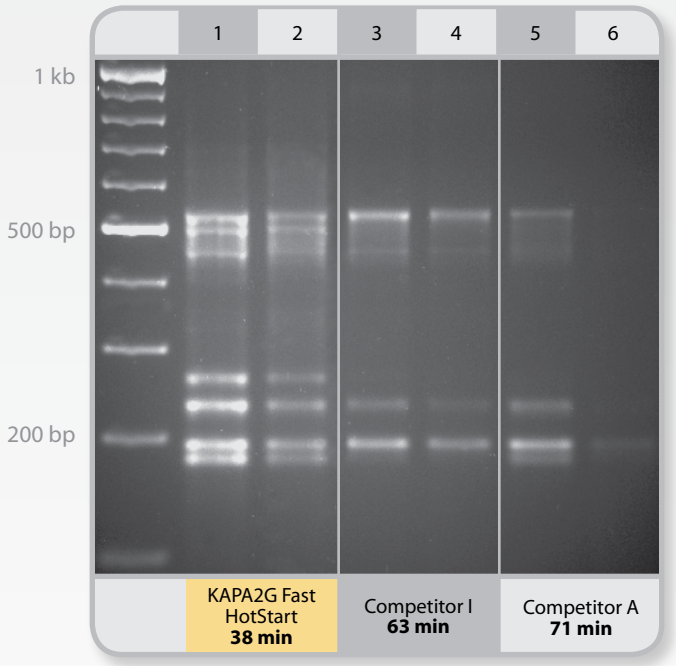
Amplification of 5 human gene fragments using KAPA2G Fast HotStart or competitor hot start Taq formulations. Reactions (25 µl) contained 5 ng human genomic DNA and 0.5 units (KAPA2G Fast HotStart or Competitor I) or 0.625 units (Competitor A or Competitor Q) enzyme. For amplicons with a GC content >65% (#2 and 3), 7.5% DMSO was included in reaction mixes. Cycling was performed on an Eppendorf Mastercycler ep*gradient* S, using 3-step cycling profiles (35 cycles) with 15 sec denaturation (95ºC) and 15 sec annealing (60ºC) per cycle for all enzymes. Extension (72ºC) was 1 sec per cycle for KAPA2G Fast HotStart and 60 sec per cycle for competitor enzymes. Initial denaturation/enzyme re-activation and final extension times used for each enzyme were as per the manufacturers recommendations. The total reaction time for each enzyme is as indicated.

The gene target, length and GC content of each amplicon are as follows: 1 = 5,10-methylene tetrahydrofolate reductase, 150 bp, 54.0% GC; 2 = guanine nucleotide binding protein, 241 bp, 83.8% GC; 3 = RET proto-oncogene, 392 bp, 79.1% GC; 4 = zinc finger protein 41, 502 bp, 42.5% GC and 5 = epidermal growth factor receptor, 626 bp, 42.0% GC.

Faster, more sensitive Multiplex PCR

With KAPA2G Fast HotStart, faster Multiplex PCR is also possible. Because of the complexity of multiplex assays, longer annealing times are maintained, but extension times may be reduced to 10 sec per cycle for fragments <1 kb. With optimized KAPA2G Fast HotStart protocols, time savings of 35 - 70% can be achieved.

Multiplex PCR assays typically employ a high template concentration. Using KAPA2G Fast HotStart in 1.5x KAPA2G HotStart buffer, improved sensitivity was achieved in all model systems tested.



Amplification of 7 fragments of the human Duchenne muscular dystrophy (DMD) locus in a Multiplex PCR using KAPA2G Fast HotStart or competitor hot start Taq formulations. Reactions (25 µl) contained 100 ng (lanes 1, 3 and 5) or 10 ng (lanes 2, 4 and 6) female human genomic DNA, 0.25 µM of each primer, 3 mM $MgCl_2$ and 1 unit enzyme. KAPA2G Fast HotStart reactions were performed in 1.5x KAPA2G HotStart Buffer A and competitor reactions 1x reaction buffer. Cycling was performed on an Eppendorf Mastercycler ep*gradient* S, using 3-step cycling profiles (30 cycles) and an initial denaturation/enzyme re-activation of 2 min for KAPA2G Fast HotStart and Competitor I and 10 min for Competitor A. For KAPA2G Fast HotStart, each cycle consisted of 15 sec denaturation (95ºC), 30 sec annealing (55ºC) and extension for 10 sec at 72ºC. For competitor enzymes, each cycle consisted of 30 sec denaturation (95ºC), 30 sec annealing (55ºC) and extension for 45 sec at 72ºC. The total reaction time for each enzyme is as indicated.

Fragments amplified in this multiplex assay are (from top to bottom): exon Pm, 535 bp; exon 48, 506 bp; exon 19, 459 bp; exon 50, 271 bp; exon 13, 238 bp; exon 4, 196 bp and exon 47, 181 bp.

KAPA2G Fast HotStart PCR kit components

- KAPA2G Fast HotStart DNA Polymerase (5 U/µl)
- 5x KAPA2G HotStart Buffer A with $MgCl_2$
- 5x KAPA2G HotStart Buffer B with $MgCl_2$
- 25 mM $MgCl_2$
- KAPA dNTP Mix (10 mM each dNTP) (KK5500 and KK5502 only)

KAPA2G Fast HotStart PCR kits

Product code	Kit Size
With dNTPs	
KK5500	500 units
KK5502	250 units
Without dNTPs	
KK5501	500 units
KK5503	250 units

Boston, Massachusetts, United States
600 West Cumming Park, Suite 5350
Woburn, MA, 01801 U.S.A.
Tel: +1 781 497 2933 Fax: +1 781 497 2934
Email: info@kapabiosystems.com

Cape Town, South Africa
Research, Development and Manufacturing Facility
2nd Floor, Old Warehouse Building, Black River Park,
Fir Road, Observatory, 7925 Cape Town, South Africa
Tel: +27 21 448 8200 Fax: +27 21 448 6503
Email: info@kapabiosystems.com

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