

# Introducing **KAPA2G™** Robust HotStart



A second generation DNA polymerase evolved for extreme robustness.

**KAPA2G Robust HotStart combines a second-generation DNA polymerase evolved for extreme robustness with HotStart technology for optimal performance.**

**The result is industry-leading performance:**

- Robust performance across a wide range of templates and amplicon types.
- Improved tolerance to common PCR inhibitors.
- Higher yield and sensitivity per unit of enzyme.
- Three novel buffers and a proprietary PCR enhancer offer extended optimization options for difficult templates.

# >> KAPA2G™ Robust HotStart

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## Product Description

KAPA2G Robust HotStart is based on KAPA2G Robust DNA Polymerase, a highly robust and versatile second-generation enzyme derived through a process of molecular evolution. The novel amino acid mutations in KAPA2G Robust DNA Polymerase offer higher processivity and specific activity, which translates to robust performance across a wide range of GC- and AT-rich templates and amplicons, as well as improved tolerance to common PCR inhibitors. 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.

KAPA2G Robust HotStart Buffers A and B and the proprietary additive, KAPAEhancer 1, offer extended optimization options for diverse and difficult templates. The kit also contains KAPA2G Robust HotStart GC Buffer, a novel buffer formulated specifically for GC-rich templates and amplicons. Like wild-type Taq, KAPA2G Robust DNA Polymerase has 5'-3' polymerase and exonuclease activities, but no 3'-5' exonuclease (proofreading activity). The fidelity of KAPA2G Robust DNA Polymerase is similar to that of wild-type Taq.

## Product Applications

KAPA2G Robust HotStart is recommended for all standard end-point PCR assays, particularly those in which wild-type Taq DNA polymerase does not perform satisfactorily. It is particularly suited for:

- Amplification from templates with a high GC- or AT content.
- Templates containing common PCR inhibitors (e.g. salts, urea, SDS and ethanol).
- Amplification from crude samples, e.g. buccal swabs, cultured mammalian, yeast or bacterial cells (Colony PCR).
- Optimization of low yield or low specificity assays.

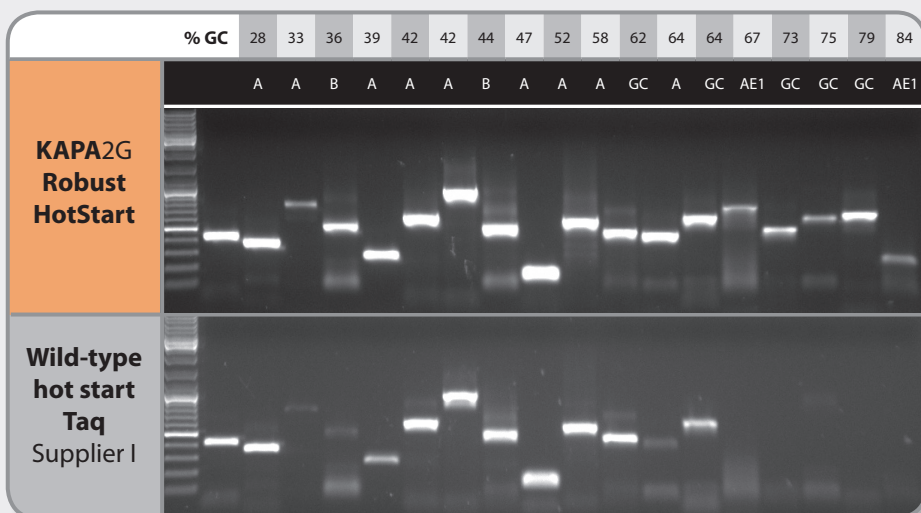
Amplicons generated with KAPA2G Robust HotStart are suitable for routine downstream applications, including restriction enzyme digestion, cloning and sequencing.

### Engineered KAPA2G Robust HotStart DNA Polymerase with novel buffers and proprietary KAPAEhancer offer:

- Robust performance across a wide range of GC- and AT-rich templates and amplicons.
- Greatly improved tolerance to common PCR inhibitors.
- Higher yield per unit of enzyme, which often translates to improved sensitivity.
- Extended options to optimize difficult assays.

## Robust performance and higher success rate with diverse amplicons

KAPA2G Robust HotStart offers robust performance and high yields across a wide range of amplicon types and sizes, and performs equally well with AT- and GC-rich amplicons. Three novel buffers and the proprietary additive, KAPAEhancer 1, offer extended optimization options and higher success rates with difficult amplicons and templates.



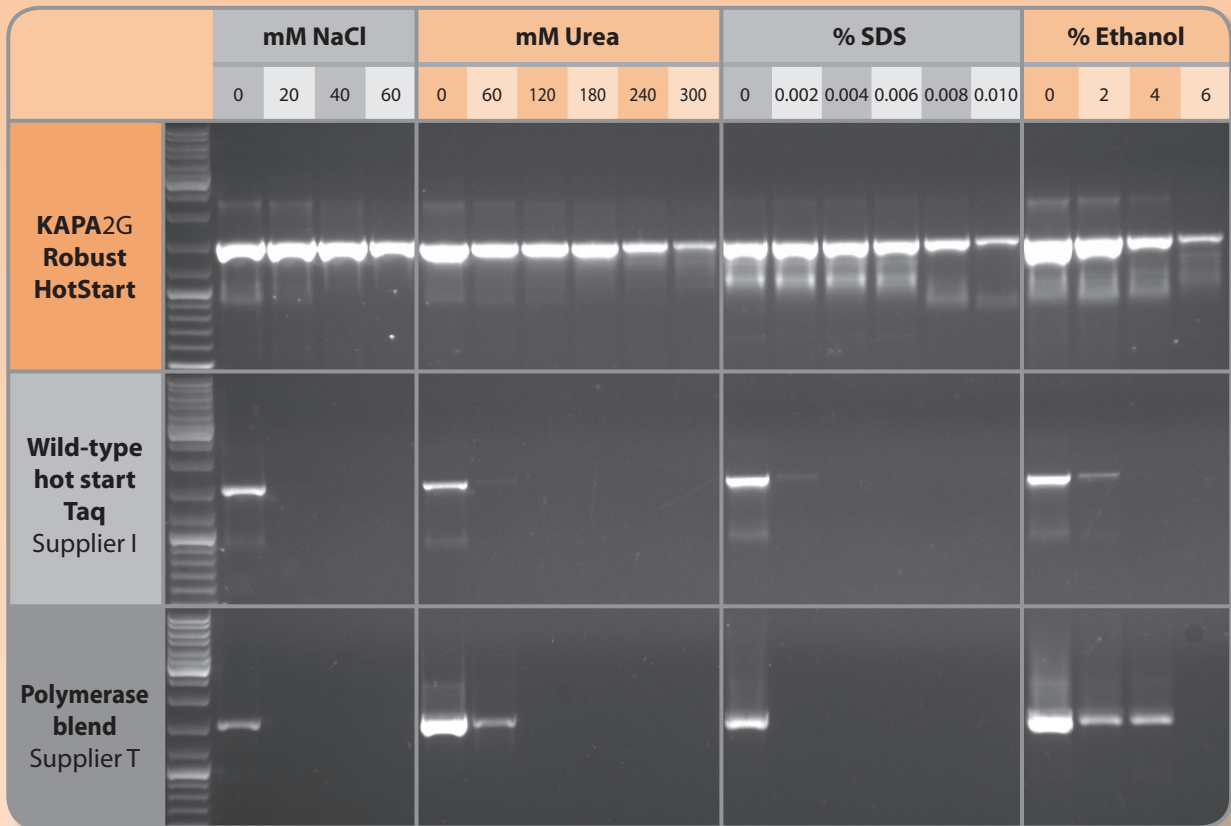
Amplification of 18 human single-copy gene fragments using the KAPA2G Robust HotStart PCR kit or wild-type hot start Taq polymerase.

Amplicons range from 0.14 to 1.0 kb in size and have a GC content between 28 and 84%. The extended optimization options provided in the KAPA2G Robust HotStart PCR kit allowed for successful amplification of all 18 amplicons: 9 with Buffer A (A), 2 with Buffer A + KAPAEhancer 1 (AE1) and 5 with GC Buffer. Less than 75% of the amplicons could be amplified with wild-type hot start Taq and standard buffer (+6% DMSO for amplicons with a GC content >60%), with very low yields in a quarter of successful reactions.

All reactions contained 10 ng human genomic DNA as template and 0.5 units of enzyme per 25 µl reaction. Cycling was performed with an Eppendorf Mastercycler epgradient S, using a standard 3-step cycling profile (35 cycles) with an annealing temperature of 60°C for all amplicons. An extension time of 30 sec/kb was used for KAPA2G Robust HotStart and 1 min/kb for wild-type hot start Taq.

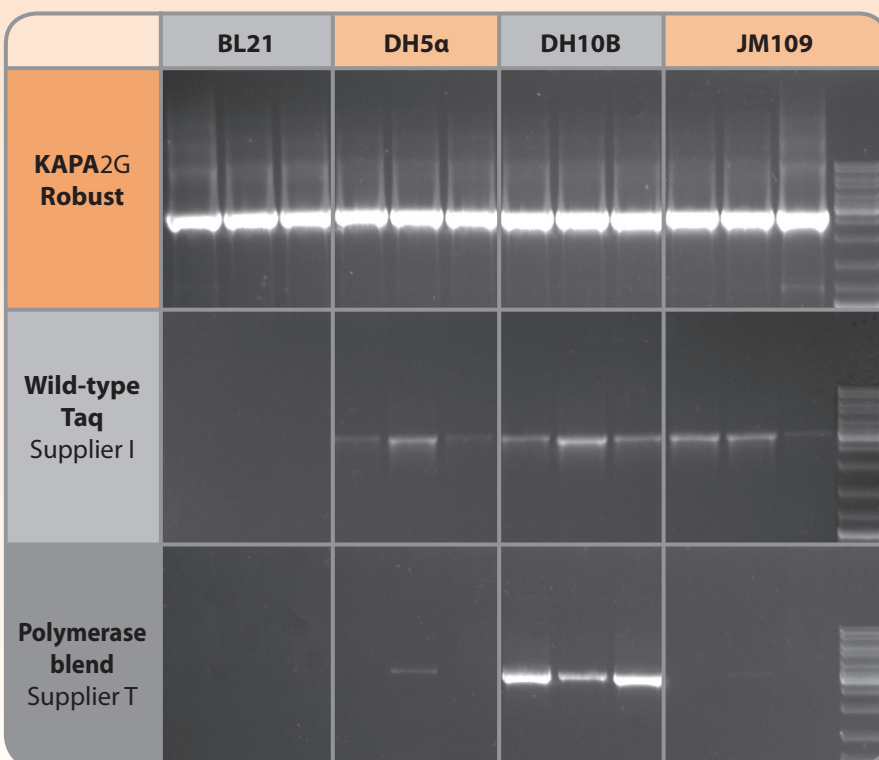
## Greatly improved tolerance to common PCR inhibitors

KAPA2G Robust HotStart was engineered for high performance in chemically complex reaction conditions. The result is superior tolerance to a wide range of common PCR inhibitors, when compared to wild-type hot start Taq polymerase and so-called “robust” polymerase blends. In addition to the examples below, KAPA2G Robust HotStart DNA Polymerase shows improved tolerance to other inhibitors, including KCl, sodium acetate, isopropanol and phenol (at high template and enzyme concentrations).



Amplification of a 1.5 kb fragment from 1 pg plasmid DNA in the presence of four common PCR inhibitors using the KAPA2G Robust HotStart PCR kit (top), wild-type hot start Taq polymerase (middle) or a “robust” blend of thermostable DNA polymerases (bottom). All reactions contained 0.5 units of enzyme per 25 µl reaction, except for reactions with the polymerase blend (0.625 units per reaction). KAPA2G Robust HotStart Buffer B was used throughout, with the addition of KAPAEhancer 1 for reactions containing SDS. Cycling was performed with an Eppendorf Mastercycler eppgradient S, using a standard 3-step cycling profile (35 cycles) with an annealing temperature of 64°C and 1.5 min extension time per cycle for all enzymes.

## Unrivalled performance in Colony PCR



The improved inhibitor tolerance of KAPA2G Robust HotStart translates into unrivalled performance in Colony PCR, a PCR application that is prone to failure and inconsistency due to the presence of inhibitors. With the KAPA2G Robust HotStart PCR kit, a 100% success rate in Colony PCR is achievable, starting from plated bacterial colonies or overnight cultures.

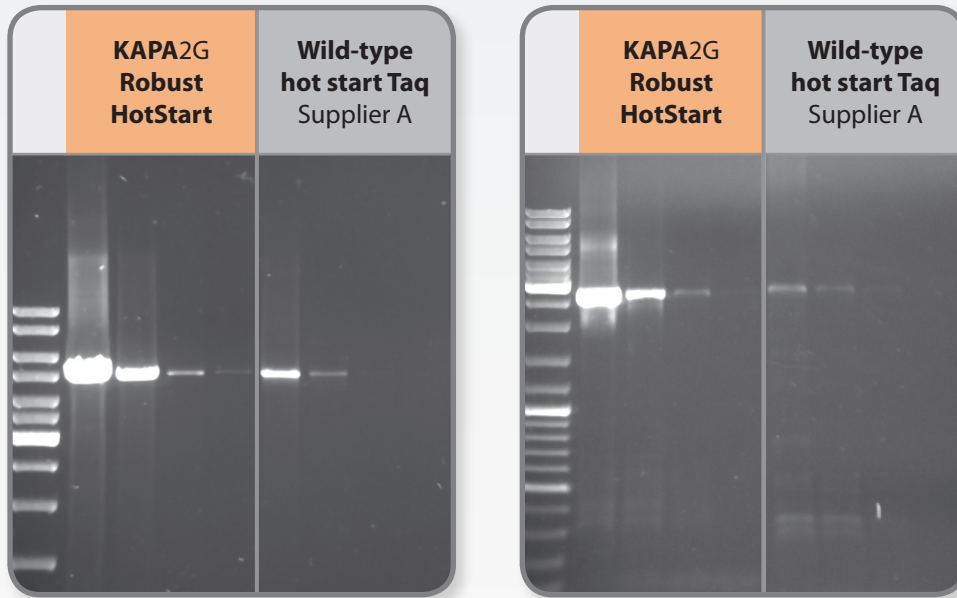
Amplification of a 2.7 kb gene from recombinant *E. coli* colonies using the KAPA2G Robust HotStart PCR Kit (top), wild-type hot start Taq (middle) or a blend of thermostable DNA polymerases (bottom).

Three single colonies from each of four commonly used *E. coli* strains (BL21, DH5α, DH10B and JM109) were picked from LB-agar + ampicillin plates and resuspended in PCR grade water. 1 µl resuspended bacterial cells was used as template in a 25 µl PCR.

All reactions contained 0.5 units of enzyme, except for reactions with the polymerase blend (0.625 units per reaction). KAPA2G Robust HotStart Buffer A was used in all KAPA2G Robust HotStart reactions. Cycling was performed with a G-Storm GS1 thermocycler with fast block, using a standard 3-step cycling profile (35 cycles) with 1 min/kb extension time for all enzymes.

## KAPA2G Robust HotStart PCR kits offer higher yields and sensitivity

The higher processivity and specific activity of KAPA2G Robust HotStart DNA Polymerase results in higher yields per unit of enzyme, which often translates into improved sensitivity. Higher yields and sensitivity are also achieved through the use of novel buffers, which have been formulated for optimal annealing specificity. For certain assays, specificity and efficiency may be improved further through the use of KAPAEhancer 1 in combination with KAPA2G Robust HotStart Buffer A or B.



Amplification of a 5 kb lambda (left) and a 2.7 kb human (right) amplicon using the KAPA2G Robust HotStart PCR Kit or wild-type hot start Taq polymerase. A 10-fold dilution series of template DNA ( $10^6$  to  $10^3$  copies lambda DNA or 30,000 to 30 copies human DNA, respectively) was included in each experiment. All reactions contained 0.5 units of enzyme per 25  $\mu$ l reaction, except for 5 kb reactions with KAPA2G Robust HotStart, in which only 0.25 units of enzyme were used. For the 2.7 kb assay, KAPAEhancer 1 was included in KAPA2G Robust HotStart reactions. Cycling was performed with a G-Storm GS1 thermocycler with a fast block (5 kb amplicon) or Eppendorf Mastercycler *epgradient* S (2.7 kb amplicon), using standard 3-step cycling profiles (35 cycles) with extension rates of 1 - 2 min/kb.

### KAPA2G Robust HotStart PCR kit components

- KAPA2G Robust HotStart DNA Polymerase (5 U/ $\mu$ l)
- 5x KAPA2G Robust HotStart Buffer A with  $MgCl_2$
- 5x KAPA2G Robust HotStart Buffer B with  $MgCl_2$
- 5x KAPA2G Robust HotStart GC Buffer with  $MgCl_2$
- 5x KAPAEhancer 1
- 25 mM  $MgCl_2$
- KAPA dNTP Mix (10 mM each dNTP) (KK5516 and KK5518 only)

#### KAPA2G Robust HotStart PCR Kits

Product code	Kit Size
<b>Without dNTPs</b>	
KK5522	100 units
KK5515	250 units
KK5517	500 units
KK5525	2,500 units
<b>With dNTPs</b>	
KK5516	250 units
KK5518	500 units

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