

KAPA2G Robust

Evolved to solve

The second-generation KAPA2G Robust DNA Polymerase was evolved to solve inconsistent amplification across a broad range of amplicon types (GC- and AT-rich). This product enables higher processivity and specific activity, which translates to robust PCR performance, high sensitivity, and improved tolerance to common inhibitors. The high performance is ideally suited for challenging PCR applications and difficult samples, eliminating the need for optimization using multiple enzymes and protocols.

Benefits include:

- Applications requiring higher yield per unit enzyme
- Colony PCR
- Tolerance to inhibitor carryover and crude sample PCR (e.g., FFPE)
- Routine PCR using the HotStart or ReadyMix formulation

Efficient amplification of GC- and AT-rich targets

- Robust performance across a wide range of GC- and AT-rich templates
- Increased PCR success rates

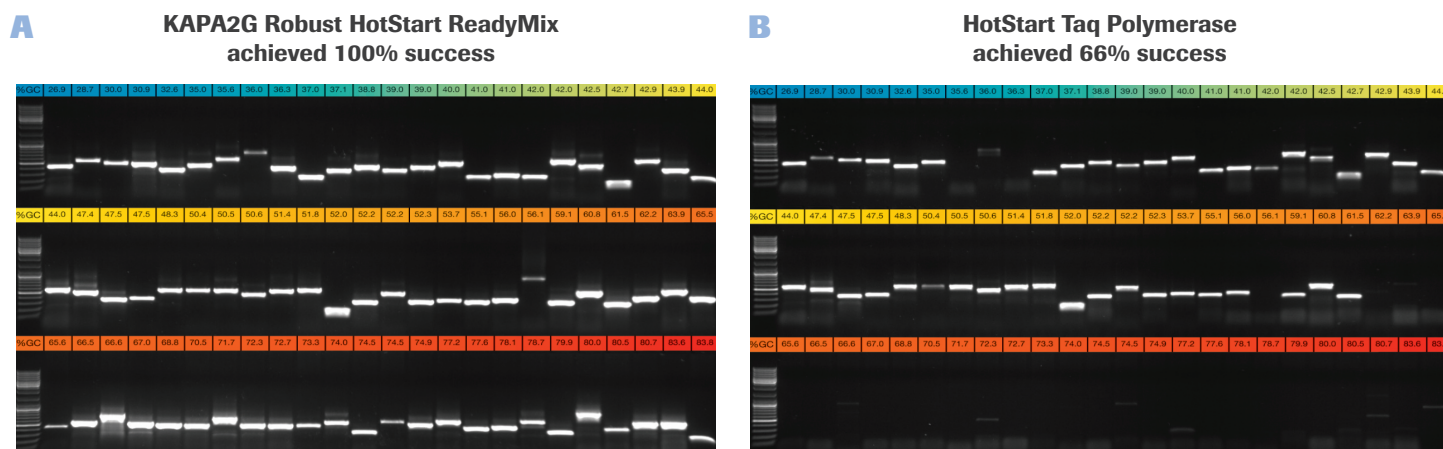


Figure 1. Increased PCR success rates. Half of each of the PCR products obtained with 72 of the 96 primer sets used in this study were electrophoresed in a 1% TBE-agarose gel. Amplicons were loaded in order of increasing GC content, with the lowest GC content (27%, blue) at the top left hand side and the highest GC content (84%, red) at the bottom right hand side of each composite gel image. Primers selected for this study had variable primer lengths, sequence composition, theoretical melting temperatures, and other design features. Some primers contained 5'-tails for post-PCR sequencing using M13 or other standard sequencing primers. **(A)** KAPA2G Robust HotStart ReadyMix reactions (25 μ L) were performed as outlined in the User Guide. **(B)** Wild-type *Taq* reactions (25 μ L, containing 0.5 U *Taq* per reaction) were performed in *Taq* reaction buffer (1.5 mM MgCl₂ at 1X), using the same final primer and dNTP concentrations as for KAPA2G Robust. All reactions contained 25 ng human genomic DNA. 5% DMSO was included in all reactions (KAPA2G Robust and *Taq*) targeting amplicons with a GC content >70%.

Improved tolerance to common PCR inhibitors

- Efficient amplification from crude samples
- Higher yield and sensitivity per unit of enzyme

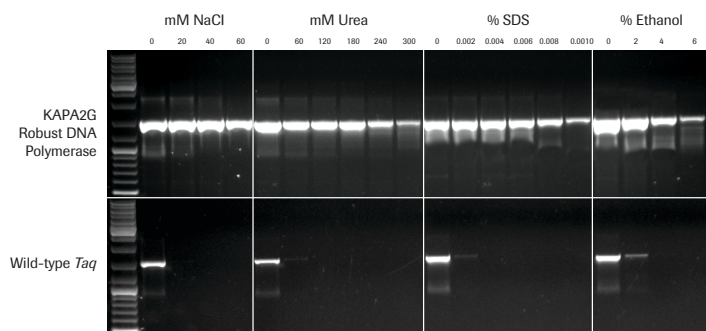


Figure 2. Amplification of a 1.5 kb fragment from 1 pg plasmid DNA in the presence of four common PCR inhibitors using the KAPA2G Robust HotStart PCR kit and wild-type hot start Taq polymerase.

All reactions contained 0.5 units of enzyme per 25 μ L reaction. KAPA2G Robust HotStart Buffer B was used throughout, with the addition of KAPAEEnhancer 1 for reactions containing SDS. Cycling was performed with an Eppendorf Mastercycler epgradient 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

- Higher yields and improved consistency of PCR direct from *E. coli* and yeast cells

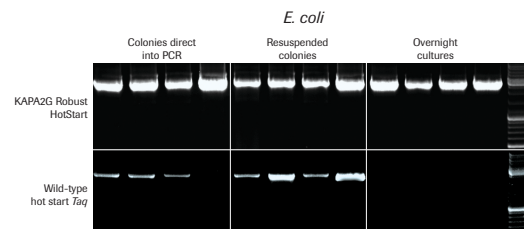


Figure 3. Amplification of a cloned 2.7 kb insert from four commonly used *E. coli* strains (DH5a, DH10B, JM109 or BL21) using KAPA2G Robust HotStart (top) or wild-type Taq (bottom). Colonies (grown on LB-agar + Amp plates) were either resuspended directly in individual PCR reactions (left) or first resuspended in PCR grade water and then added to PCR reaction mixes (middle). For overnight cultures (prepared in LB + Amp), 1 μ L was added directly to the PCR mix (right).

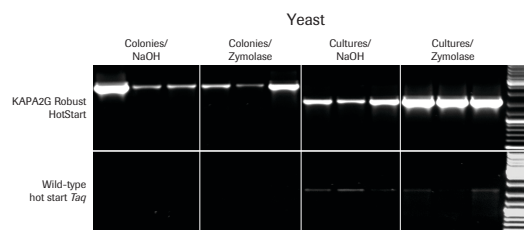


Figure 4. Amplification of a 2.5 kb (left) or 1.6 kb (right) fragment from the GSH1 gene from three commonly used *S. cerevisiae* strains (BY4742, FY23 and W303) using KAPA2G Robust HotStart (top) or wild-type Taq (bottom). Colonies (from YPD-agar plates) or YPD overnight cultures were first lysed in 50 μ L volumes with NaOH or Zymolase (as indicated).

Ordering information

Roche cat. no.	KAPA code	Description	Kit size
07960859001	KK5023	KAPA2G Robust	100 units
07960867001	KK5024	KAPA2G Robust	250 units
07960743001	KK5004	KAPA2G Robust + KAPA dNTP Mix	100 units
07960751001	KK5005	KAPA2G Robust + KAPA dNTP Mix	250 units
07961103001	KK5522	KAPA2G Robust HotStart	100 units
07961057001	KK5515	KAPA2G Robust HotStart	250 units
07961073001	KK5517	KAPA2G Robust HotStart	500 units
07961120001	KK5525	KAPA2G Robust HotStart	2500 units
07961197001	KK5532	KAPA2G Robust HotStart + KAPA dNTP Mix	100 units
07961065001	KK5516	KAPA2G Robust HotStart + KAPA dNTP Mix	250 units
07961081001	KK5518	KAPA2G Robust HotStart + KAPA dNTP Mix	500 units
07961332001	KK5701	KAPA2G Robust HotStart Ready Mix	100 rxns
07961359001	KK5702	KAPA2G Robust HotStart Ready Mix	500 rxns

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