Introducing **KAPA**2G™ Robust PCR Kits

A second generation DNA polymerase engineered for extreme robustness.

KAPA2G Robust PCR Kits contain a second generation DNA polymerase engineered for extreme robustness.

The result is industry-leading performance:

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

KAPABIOSYSTEMS
www.kapabiosystems.com

next generation thinking
in enzyme technology

>> KAPA2G™ Robust PCR Kit

A second generation DNA polymerase engineered for extreme robustness.

Engineered KAPA2G Robust DNA Polymerase plus optimized buffers and proprietary KAPAEnhancers 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 .

Product Description

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

The kit is based on KAPA2G Robust DNA Polymerase, a highly robust and versatile engineered enzyme that was derived from Taq DNA polymerase using the KAPA Biosystems molecular evolution platform. 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. Two proprietary PCR enhancers supplied with the two high-yield KAPA2G buffers offer extended optimization options for difficult amplicons or templates.

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 the same as that of wild-type Taq.

Product Applications

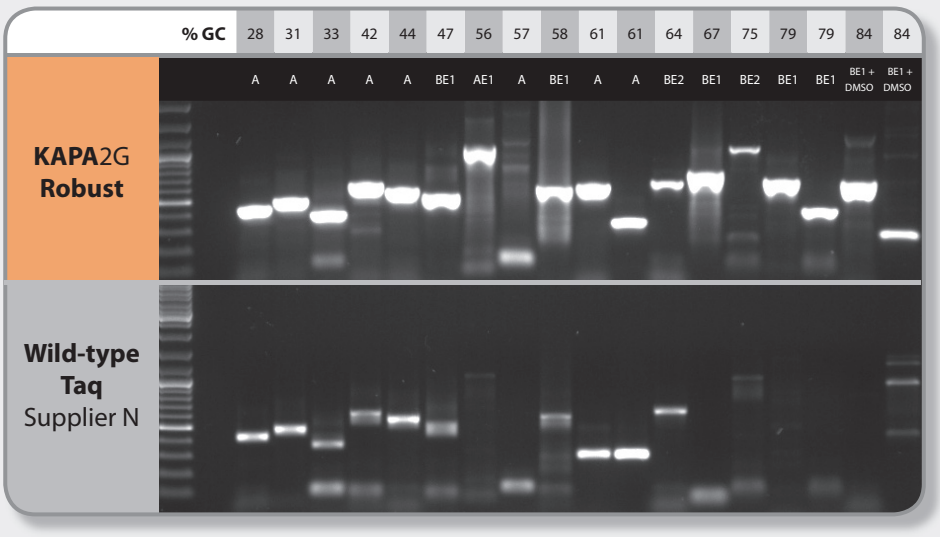
KAPA2G Robust PCR Kits are designed for the amplification of DNA fragments up to 6 kb in standard end-point PCR assays from a wide variety of templates. It is particularly suited for:

- Amplification from templates with a high GC- or AT content
- Templates containing common PCR inhibitors (e.g. salts, urea, SDS, ethanol or EDTA) at levels inhibitory to wild-type Taq
- Colony PCR
- Optimization of low yield or low specificity assays using KAPAEnhancer 1 or 2

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

Robust performance with difficult templates

The KAPA2G Robust PCR Kit offers robust performance and high yield across a wide range of amplicon types and sizes, and performs equally well with AT- and GC-rich amplicons. In combination with KAPAEnhancers 1 and 2, the two high-yield KAPA2G buffers supplied in the kit offer 8 optimization options for difficult amplicons or templates. In addition, KAPA2G Robust DNA Polymerase is compatible with other commonly used PCR additives, such as DMSO, betaine and BSA.



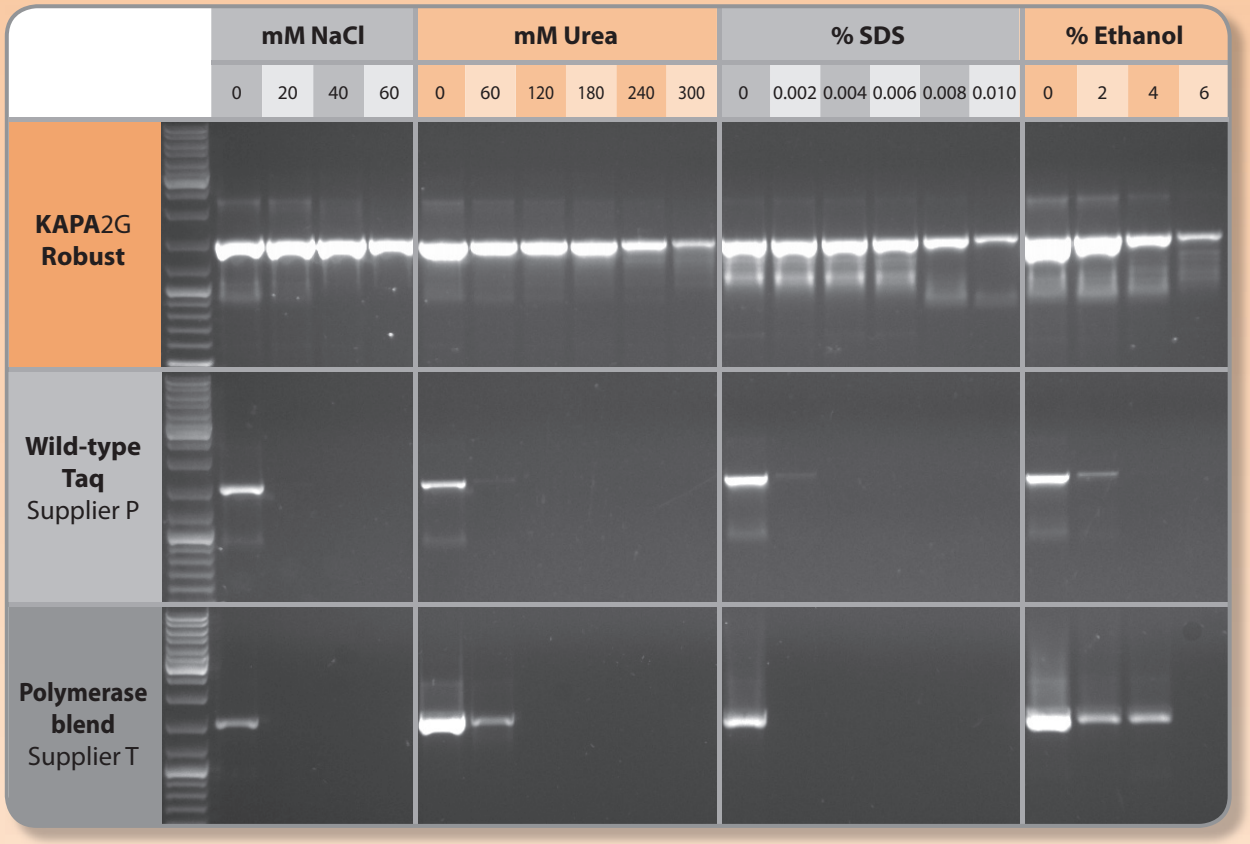
Amplification of 18 different human single-copy gene fragments using the KAPA2G Robust PCR Kit or wild-type Taq polymerase.

Amplicons range from 0.1 to 1.0 kb in size and have a GC content between 28 and 84%. The extended optimization options provided in the KAPA2G Robust PCR Kit allowed for successful amplification of all 18 amplicons: 8 with KAPA2G Buffer A (A), 6 with KAPA2G Buffer A or B + KAPAEnhancer 1 (AE1 or BE1), 2 with KAPA Buffer B + KAPAEnhancer 2 (BE2) and the 2 amplicons with a GC content >80% with KAPA2G Buffer B + KAPAEnhancer 1 + 5% DMSO. Only half of the amplicons could be amplified with a standard wild-type Taq polymerase kit.

All reactions contained 20 ng human genomic DNA as template and 0.5 units of enzyme per 25 µl reaction. Cycling was performed with an Eppendorf Mastercycler ep*gradient* S, using a standard 3-step cycling profile (35 cycles) with an annealing temperature of 60ºC for all amplicons.

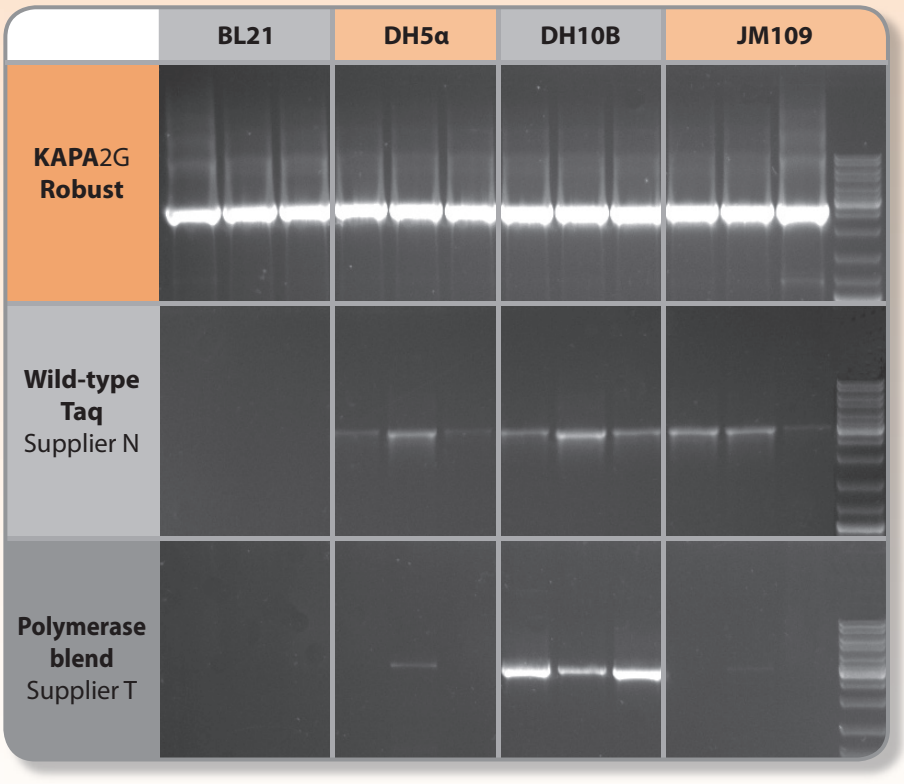
Greatly improved tolerance to common PCR inhibitors

KAPA2G Robust DNA Polymerase 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 Taq polymerase and so-called "robust" polymerase blends. In addition to the examples below, KAPA2G Robust 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 PCR Kit (top), wild-type 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 Buffer A was used for the urea assay, Buffer B for the NaCl and ethanol assays, and Buffer B + KAPAEnhancer 1 for the SDS assay. Cycling was performed with an Eppendorf Mastercycler ep*gradient* S, using a standard 3-step cycling profile (35 cycles) with an annealing temperature of 64ºC and 1.5 min extension time per cycle.

Unrivalled performance in Colony PCR



The improved inhibitor tolerance of KAPA2G Robust DNA Polymerase 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 PCR Kit, a 100% success rate in Colony PCR is achievable, starting from plated bacterial colonies or overnight cultures.

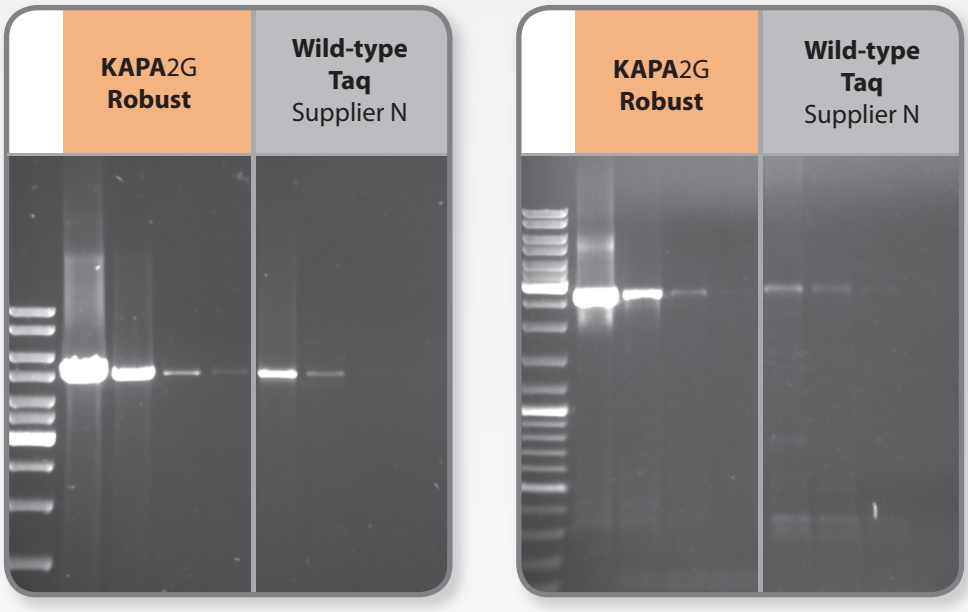
Amplification of a 2.7 kb gene from recombinant *E. coli* colonies using the KAPA2G Robust PCR Kit (top), wild-type Taq (middle) or a blend of thermostable DNA polymerases (bottom).

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 Buffer B was used in all KAPA2G Robust reactions. Cycling was performed with a G-Storm GS1 thermocycler with fast block, using a standard 3-step cycling profile (35 cycles) with 30 sec/kb extension time.

KAPA2G Robust PCR Kits offer higher yield and sensitivity

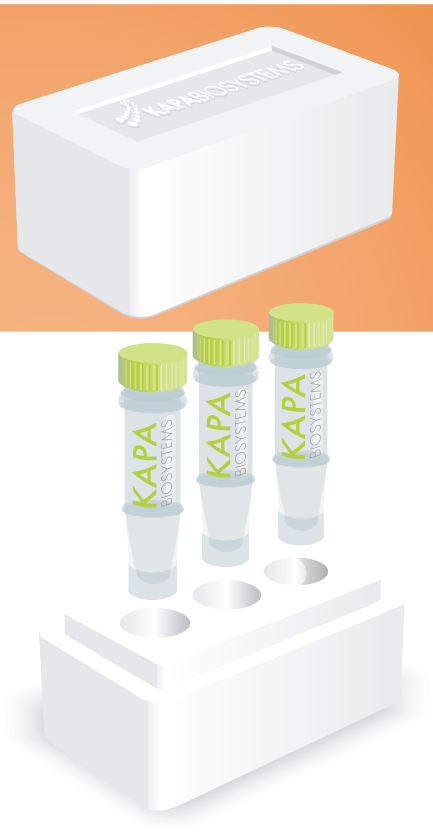
The higher specific activity of KAPA2G Robust DNA Polymerase offers higher yields per unit of enzyme, which often translates into improved sensitivity. This is particularly relevant for larger amplicons (3 - 5 kb), where wild-type Taq typically performs poorly. Assays suffering from low efficiency due to low specificity may be improved significantly through the use of KAPA2G Robust DNA Polymerase in combination with KAPAEnhancer 1 or 2.



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

KAPA2G Robust PCR Kit Components

- KAPA2G Robust DNA Polymerase (5 U/µl)
- 5x KAPA2G Buffer A
- 5x KAPA2G Buffer B
- 25 mM $MgCl_2$
- 5x KAPAEnhancer 1
- 25x KAPAEnhancer 2
- KAPA dNTP Mix (10 mM each nucleotide)



KAPA2G Robust PCR Kits	
	Kit Size
Without loading dye	
KK5004	100 units
KK5005	250 units
With loading dye	
KK5006	100 units
KK5007	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

KAPABIOSYSTEMS
www.kapabiosystems.com