



## KAPA™ SYBR® FAST qPCR Kit Master Mix (2X) ABI Prism™

### 1. Product Description

KAPA SYBR® FAST qPCR Master Mix is designed for high performance, high-throughput, real-time PCR. The kit contains a novel DNA polymerase engineered through a process of molecular evolution. The result is a unique enzyme, specifically designed for qPCR using SYBR® Green I dye chemistry.

KAPA SYBR® DNA Polymerase has been engineered to perform optimally in stringent real-time quantitative PCR (qPCR) reaction conditions, exhibiting dramatic improvements to signal-to-noise ratio (fluorescence), cycle threshold ( $C_T$ ), linearity, and sensitivity. The KAPA SYBR® DNA Polymerase and proprietary buffer system enhances the amplification efficiency of difficult templates, including both GC-rich and AT-rich templates.

KAPA SYBR® FAST qPCR Master Mix (2X) ABI Prism™ is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR on ABI real-time instruments that support normalization with ROX reference dye at a final concentration of 500 nM. The KAPA SYBR® FAST qPCR Kit is supplied as a 2X Master Mix with integrated antibody-mediated hot start, SYBR® Green I fluorescent dye, ROX reference dye,  $MgCl_2$ , dNTPs, and stabilizers.

### 2. Product Applications

KAPA SYBR® FAST qPCR Kits are ideally suited for:

- Gene expression analysis
- Low copy gene detection
- Microarray validation
- Gene knockdown validation

### 3. Product Specifications

#### 3.1 Shipping and Storage

Upon arrival, store kit components protected from light at  $-20\text{ }^\circ\text{C}$  in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the master mix is retained for 18 months from the date of receipt.

#### 3.2 Handling

Minimize exposure of the Master Mix (2X) to direct light. Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity. Always ensure that the product has been fully thawed and mixed before use.

#### 3.3 Quality Control

KAPA SYBR® FAST qPCR Master Mix (2X) is free of contaminating DNase and RNase. It is functionally tested to demonstrate resolution of 5 orders of linear dynamic range using human genomic DNA as template and *B-actin* primers.

#### 3.4 Product Use Limitations

KAPA SYBR® FAST qPCR Master Mix (2X) is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.

<b>KK4603</b> 100 reactions in 20 $\mu\text{l}$ volume	<b>KAPA SYBR® FAST Master Mix (2X) ABI Prism™ 1 x 1 ml</b> Contains: - qPCR Master Mix (2X) with ROX Reference Dye
<b>KK4604</b> 500 reactions in 20 $\mu\text{l}$ volume	<b>KAPA SYBR® FAST Master Mix (2X) ABI Prism™ 1 x 5 ml</b> Contains: - qPCR Master Mix (2X) with ROX Reference Dye
<b>KK4605</b> 1000 reactions in 20 $\mu\text{l}$ volume	<b>KAPA SYBR® FAST Master Mix (2X) ABI Prism™ 2 x 5 ml</b> Contains: - qPCR Master Mix (2X) with ROX Reference Dye

The final  $MgCl_2$  concentration per reaction is 2.5mM

#### Quick Notes

- This kit contains a highly engineered enzyme optimized for use in qPCR using SYBR® Green I dye chemistry.
- The 2X Master Mix contains a proprietary buffer that together with the novel enzyme enhances the amplification efficiency of both high GC and high AT templates.
- 20 sec initial denaturation at  $95\text{ }^\circ\text{C}$  is sufficient for enzyme reactivation, however optimal denaturation of complex targets may require up to 3 min denaturation.
- For two-step cycling, use 20 sec combined annealing/extension/ data acquisition.
- Do not exceed 25  $\mu\text{l}$  reaction volumes.

#### Instrument Table

Instrument	ROX Reference dye
ABI 5700, 7000, 7300, 7700, and 7900HT StepOne™, StepOnePlus™ and ViiA™ 7	500 nM final



## 4. KAPA SYBR® FAST qPCR Protocol

Any existing qPCR assay performed efficiently using standard cycling conditions may be converted to a Fast qPCR assay with KAPA SYBR® FAST qPCR Kits. Typically, minimal re-optimization of reaction parameters is required.

This protocol is intended for use with the ABI PRISM®7000, 7700, 7900HT, the ABI 7300 Real-Time PCR Systems, the GeneAmp®5700, and the StepOne™, and StepOnePlus™. This kit is not compatible with instruments that use ROX at a final concentration lower than 500 nM.

### 4.1 Step 1: qPCR Reaction Setup

- Before preparing qPCR reactions, thoroughly mix the KAPA SYBR® FAST qPCR Master Mix (2X), template DNA, and primers.
- Calculate the required volumes of each component based on the following table:

	Final concentration	20 µl rxn
PCR grade water up to 20 µl		As required
KAPA SYBR® FAST qPCR Master Mix (2X) ABI Prism™	1X	10 µl
Forward Primer (10 µM)	200 nM	0.4 µl
Reverse Primer (10 µM)	200 nM	0.4 µl
Template DNA	(<20 ng/20 µl rxn)	Variable

### 4.2 Step 2: Plate Setup

- Transfer the appropriate volume of reaction mixture to each well of a PCR tube/plate. Reaction volumes may be scaled down from 20 µl to 10 µl if low volume tubes/plates are used.
- Cap or seal the reaction tube/plate and centrifuge briefly.

### 4.3 Step 3: Run the qPCR Reaction

- If applicable, select fast mode on the instrument.
- Program the following cycling protocol:

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	20 sec - 3 min*	Hold
Denature	95 °C	1 - 3 sec	40
Anneal/extend***	60 °C	≥ 20 sec**	
Dissociation	According to instrument guidelines		

\*20 sec at 95 °C is sufficient time for enzyme activation, however optimal denaturation of complex targets may require up to 3 min denaturation.

\*\*Select minimum time (not less than 20 sec) according to instrument user guide.

\*\*\*For 3 step cycling protocols, anneal at optimal annealing temperature for 20 sec followed by the minimum time required for data acquisition at 72 °C according to instrument user guide.

### 4.4 Step 4: Analyze the results

- Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.



## 5. Important Parameters

### 5.1 Template

Genomic DNA, plasmid DNA, or cDNA can be used as template. For optimal quantitative results use up to 20 ng of genomic DNA or plasmid DNA per 20 µl reaction (for smaller volumes, the amount of template should be decreased equivalently). Using greater amounts of template may reduce the maximum fluorescence signal and linearity of standard curves due to binding of the SYBR® Green I dye to the template. For two-step RT-PCR, use either undiluted or diluted cDNA generated from up to 1 µg of total RNA. The volume of the cDNA added (from the RT reaction) should not exceed 10% of the final PCR volume (e.g., for a 20 µl qPCR reaction, use up to 2.0 µl of undiluted cDNA).

### 5.2 Primers

Careful primer design and purification (HPLC-purified primers are recommended) is particularly important in order to minimize loss in sensitivity due to the production of nonspecific amplification products in SYBR® Green I-based qPCR. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the PCR reaction (50 - 400 nM of each primer). For optimal results, design primers that amplify PCR products 60 - 400 bp in length. The primers should exhibit a melting temperature ( $T_m$ ) of approximately 60 °C, to take advantage of two-step cycling. If performing real-time two-step RT-PCR, we recommend designing primers specifically for amplification of cDNA derived from mRNA. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

### 5.3 KAPA SYBR® DNA Polymerase

KAPA SYBR® DNA polymerase is a highly engineered version of Taq DNA polymerase designed specifically for real-time qPCR. KAPA SYBR® DNA Polymerase displays no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer-dimers during reaction setup and the first denaturation step, resulting in high PCR specificity and accurate quantification. The enzyme is activated at the start of a reaction by a 20 sec, 95 °C incubation step. The activation of the enzyme is complete after 20 sec, however complex targets may require up to 3 min for optimal denaturation. The hot start feature enables reactions to be set up rapidly and conveniently at room temperature.

### 5.4 Melting Curve Analysis

Following real-time qPCR, melting curve analysis should always be performed to identify the presence of primer-dimers and analyze the specificity of the reaction. Program your thermocycler according to the instructions provided.

### 5.5 SYBR® Green I

KAPA SYBR® FAST qPCR Master Mix (2X) contains an elevated, optimized concentration of the fluorescent dye, SYBR® Green I. High signal intensities are achieved as a result of increased tolerance to high concentrations of SYBR® Green I by the engineered, novel KAPA SYBR® DNA Polymerase. SYBR® Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The excitation and emission maxima of SYBR® Green I are at 494 nm and 521 nm, respectively, which are compatible with use on any real-time cycler.

### 5.6 Magnesium chloride

The  $MgCl_2$  concentration in KAPA SYBR® FAST qPCR Master Mix (2X) is optimized for most primer combinations. You do not need to add additional  $MgCl_2$  to the mix to get efficient and specific PCR.



## 6. Troubleshooting

Symptom	Possible Cause	Solution
High baseline fluorescence No product on either qPCR graph or on a gel	Starting amount of template is too high The protocol was not followed correctly  Template contains inhibitors Primer design incorrect or annealing temperature too high	Reduce the amount of template in the reaction.  Verify that all the steps have been followed and the correct reagents, dilutions, volumes, detection format, and cycling parameters have been used. Re-purify or re-isolate your template. Verify primer selection. Lower the annealing temperature in 2 °C increments.
Product detected later than expected	Amplicon length is too long PCR annealing/extension time is too short for optimal performance MgCl <sub>2</sub> concentration adjusted	Optimal results are obtained with amplicons of 60 - 400 bp or less. Verify that the correct cycling protocol has been used.  Do not adjust the MgCl <sub>2</sub> concentration of KAPA SYBR® qPCR Master Mix (2X).
Poor low copy number sensitivity	Primer design or annealing temperature sub-optimal	Redesign primers. HPLC purification of primers greatly reduces primer-dimer problems and increases sensitivity. Adjust primer concentration and T <sub>m</sub> . Ensure correct cycling parameters.
Low fluorescence intensity	Incorrect handling of samples	SYBR® Green I dye is light sensitive; avoid exposure to light and repeated freeze-thaw cycles.
Increased signal in no DNA control	One of the reagents has been contaminated  Primer-dimer formation	Take standard precautions to avoid contamination when preparing your PCR reactions. Ideally, amplification reactions should be set up in a DNA-free environment using aerosol-resistant barrier tips. Redesign primers. HPLC purification of primers greatly reduces primer-dimer problems and increase sensitivity. Adjust primer concentration and T <sub>m</sub> .
Melting temperature of a product varies from experiment to experiment	Variations in reaction mixture (e.g. salt)	Check the purity of the template solution.
Double melting peak appears for one product	Two products of the same length or non-uniform GC distribution in a single amplicon	Check the products on an agarose gel. Redesign primers to a region containing a uniform distribution of nucleotides (i.e., no GC hot-spots).

## 7. Note to Purchaser: Limited License

Certain applications of this product are covered by patents issued to parties other than Kapa Biosystems and applicable in certain countries. Purchase of this product does not include a license to perform any such applications. Users of this product may therefore be required to obtain a patent license depending upon the particular application and country in which the product is used.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,994,056, 6,171,785, and 5,928,907 (claim numbers 12-24, 27-28). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as apparatus or system claims in US Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

This product is provided under an agreement between Molecular Probes, Inc. and Kapa Biosystems Inc., and the manufacture, use, sale or import of this product is subject to one or more of U.S. Patent Nos. 5,436,134; 5,658,751 and corresponding international equivalents, owned by Molecular Probes, Inc. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851.

Licensed under U.S. Patent nos. 5,338,671 and 5,587,287 and corresponding patents in other countries.

The purchase of this product includes a limited, non-transferable license under specific claims of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, owned by the University of Utah Research Foundation or Evotec Biosystems GmbH and licensed to Idaho Technology, Inc. and Roche Diagnostics GmbH, to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, other than for the amount of product contained herein.

SYBR® is a registered trademark of Molecular Probes, Inc, Oregon. PRISM®, StepOne™, StepOnePlus™, ViiA™ 7, and GeneAmp® are registered trademarks of Applied Biosystems Corporation.

**For technical support please contact:**  
[support@kapabiosystems.com](mailto:support@kapabiosystems.com)

### Boston, Massachusetts, United States

600 West Cummings Park, Suite 2250  
Woburn, MA, 01801 U.S.A.  
Tel: +1 781 497 2933 Fax: +1 781 497 2934  
Email: [info@kapabiosystems.com](mailto:info@kapabiosystems.com)

### Cape Town, South Africa

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](mailto:info@kapabiosystems.com)