



# KAPA™ SYBR® FAST qPCR Kit Master Mix (2x) Universal

## 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) Universal is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR. The KAPA SYBR® FAST qPCR Kit is supplied as a 2x Master Mix with integrated antibody mediated hot start, SYBR® Green I fluorescent dye, MgCl<sub>2</sub>, dNTPs, and stabilizers. Rox reference dye is not included in the 2x Master Mix but is supplied separately.

## 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°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the master mix is retained for 6 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.

### KK4600

100 reactions in 20 µl volume

### KAPA SYBR® FAST Master Mix (2x) Universal 1 x 1ml

Contains:  
- qPCR Master Mix (2x)  
- Rox Reference Dye High (50x)\* 1 x 200 ul  
- Rox Reference Dye Low (50x)\* 1 x 200 ul

### KK4601

500 reactions in 20 µl volume

### KAPA SYBR® FAST Master Mix (2x) Universal 1 x 5ml

Contains:  
- qPCR Master Mix (2x)  
- Rox Reference Dye High (50x)\* 1 x 200 ul  
- Rox Reference Dye Low (50x)\* 1 x 200 ul

### KK4602

1000 reactions in 20 µl volume

### KAPA SYBR® FAST Master Mix (2x) Universal 2 x 5ml

Contains:  
- qPCR Master Mix (2x)  
- Rox Reference Dye High (50x)\* 2 x 200 ul  
- Rox Reference Dye Low (50x)\* 2 x 200 ul

\* See Section 5.5 for details  
The final MgCl<sub>2</sub> 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°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.
- For three-step cycling, use 20 sec for primer annealing and 1 sec for extension/data acquisition.
- Do not exceed 25ul reaction volumes.

## Instrument Table

Instrument	Rox Reference dye
ABI 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	Rox High
ABI 7500 Stratagene Mx3000P®, Mx3005P™, and Mx4000®	Rox Low
Rotor-Gene™; DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector; Mastecycler® ep realplex, Smart Cycler®, Roche LightCycler® 480, Bio-Rad CFX96	No Rox



### 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 but not limited to: ABI PRISM®7000, 7700, and 7900HT; the ABI 5700, ABI 7300 and 7500 Real-Time PCR Systems, the Stratagene Mx3000P®, Mx3005P™, and Mx4000®, the Corbett Research Rotor-Gene™, the MJ Research DNA Engine Opticon™, Opticon®2, and Chromo4™ Real-Time Detector, Eppendorf Mastercycler® eprealplex, Roche LightCycler®480, Bio-Rad CFX96, and the Cepheid Smart Cycler®.

#### 4.1 Step 1: qPCR Reaction Setup

- Before preparing qPCR reactions, thoroughly mix the KAPA SYBR® FAST qPCR Master Mix (2x), Rox Reference Dye High/Low, 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) Universal	1x	10 µl
Forward Primer (10 µM)	200nM	0.4 µl
Reverse Primer (10 µM)	200nM	0.4 µl
Template DNA	(<20 ng/20 µl rxn)	Variable
Rox High/Low (optional)	See section 5.5	0.4 µl

#### 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 1 sec extension and data acquisition at 72°C.

#### 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 (50-400nM 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 Rox Reference Dye

For certain real-time cyclers, the presence of Rox reference dye in real-time PCR compensates for non-PCR-related variations in fluorescence detection. Fluorescence from Rox reference dye does not change during the course of real-time PCR, but provides a stable baseline against which PCR-related fluorescent signals are normalized. Thus, Rox dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or differences in well position. The use of Rox dye is necessary for all Applied Biosystems instruments and is optional for the Stratagene Mx3000P®, Mx3005P™, and Mx4000® cyclers. Bio-Rad/MJ Research, Cepheid, Corbett Research, Eppendorf, and Roche instruments do not require Rox dye. The presence of Rox dye in the master mix does not interfere with real-time PCR on any instrument, since the dye is not involved in the reaction and has an emission spectrum different from that of SYBR® Green I.

Use the following table to determine the amount of Rox to use with a particular instrument:

Instrument	Amount of Rox per 20 µl reaction	
	Rox High (50x)	Rox Low (50x)
ABI 5700, 7000, 7300, 7000, 7900HT StepOne™, and StepOnePlus™	0.4 µl*	
Stratagene Mx3000P®, Mx3005P™, and Mx4000®		0.4 µl**

\*Final concentration is 500 nM \*\*Final concentration 50 nM

### 5.6 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.

### 5.7 Magnesium chloride

The MgCl<sub>2</sub> concentration in KAPA SYBR® FAST qPCR Master Mix (2x) is optimized for most primer combinations. You do not need to add additional MgCl<sub>2</sub> to the mix to get efficient and specific PCR.



# KAPA SYBR® FAST qPCR Kit

## Master Mix (2x) Universal

### 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. This kit requires a minimum of 20 sec annealing and 40 sec extension for optimal performance. 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. This kit requires a minimum of 20 sec annealing/extension or 20 sec annealing followed by 1 sec extension for 3 step protocols. 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 (ie., no GC hot-spots).

### 7. Licenses

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 to a third party or otherwise use this product or its components or 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.

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