



KAPA™ SYBR® FAST qPCR Kit

Master Mix (2X) optimized for LightCycler® 480

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) optimized for the LightCycler® 480 is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR on the LightCycler® 480 real-time PCR detection system. The KAPA SYBR® FAST qPCR Kit is supplied as a 2X Master Mix with integrated antibody-mediated hot start, SYBR® Green I fluorescent dye, $MgCl_2$, 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.

KK4609

100 reactions in 20 μl volume

KAPA SYBR® FAST Master Mix (2X)
Optimized for LightCycler® 480
1 x 1 ml

KK4610

500 reactions in 20 μl volume

KAPA SYBR® FAST Master Mix (2X)
Optimized for LightCycler® 480
1 x 5 ml

KK4611

1000 reactions in 20 μl volume

KAPA SYBR® FAST Master Mix (2X)
Optimized for LightCycler® 480
2 x 5 ml

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 three-step cycling, use 20 sec for primer annealing and 1 sec for extension/data acquisition.
- Do not exceed 25 μl reaction volumes.

Instrument Table

Instrument

LightCycler® 480



KAPA SYBR® FAST qPCR Kit

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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 LightCycler® 480 real-time PCR detection system.

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)	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

- Program the following cycling protocol:

Detection Format	Block Type	Reaction Volume	
SYBR® Green	96 well	10 - 25 µl	
	384 well	3 - 20 µl	
Program Name	Cycles	Analysis Mode	
Pre-Incubation	1	None	
Amplification	40 ¹	Quantification	
Melting Curve	1	Melting curves	
Cooling	1	None	
Program Name	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)
Pre-Incubation	95	None	00:03:00 ²
Amplification	95	None	00:00:10
	Primer dependent ³	None	00:00:20 ⁴
	72	Single	00:00:01 ⁵
Melting Curve	95	None	00:00:05
	65	None	00:01:00
	97	Continuous	5 - 10 Acquisitions/°C
Cooling	40	None	00:00:10

1. 40 cycles are suitable for most assays, however this may be reduced depending on initial target concentration.

2. 20 sec at 95 °C is sufficient time for enzyme activation, however optimal denaturation of complex targets may require up to 3 min pre-incubation at 95 °C.

3. qPCR primers are typically designed for optimal annealing at 60 °C, however optimal annealing temperatures may differ from calculated values.

4. It is not recommended to use less than 20 sec for primer annealing.

5. Due to the high processivity of the engineered KAPA SYBR® DNA Polymerase, only 1 sec at 72 °C is sufficient time for extension of amplicons <400 bp.



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.



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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 1 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 ₂ concentration adjusted	Optimal results are obtained with amplicons of 60 - 400 bp or less. This kit requires a minimum of 20 sec annealing followed by 1 sec extension for 3 step protocols. Do not adjust the MgCl ₂ 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 _m . 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 _m .
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

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