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

KR0390 - v7.12

Product Description

KAPA SYBR® FAST qPCR Master Mix is designed for high performance real-time PCR. The kit contains a novel DNA polymerase engineered via 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 in signal-to-noise ratio (fluorescence), quantitative cycle (C_q), linearity, and sensitivity. The KAPA SYBR® DNA Polymerase and proprietary buffer system improves the amplification efficiency of difficult targets, including both GC- 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 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.

Product Applications

KAPA SYBR® FAST qPCR Kits are ideally suited for:

- Gene expression analysis
- Low copy gene detection
- Absolute quantification of NGS libraries

Product Specifications

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, the master mix will retain full activity until the expiry date indicated on the kit label.

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.

Kit Codes and Components	
KK4603 100 x 20 µl reactions	**KAPA SYBR® FAST Master Mix (2X) ABI Prism™** qPCR Master Mix (2X) - 1 x 1 ml with ROX reference dye
KK4604 500 x 20 µl reactions	**KAPA SYBR® FAST Master Mix (2X) ABI Prism™** qPCR Master Mix (2X) - 1 x 5 ml with ROX reference dye
KK4605 1000 x 20 µl reactions	**KAPA SYBR® FAST Master Mix (2X) ABI Prism™** qPCR Master Mix (2X) - 1 x 5 ml with ROX reference dye

Quick Notes

- This kit contains an engineered enzyme optimized for qPCR using SYBR® Green I dye chemistry.
- The 2X Master Mix contains a proprietary buffer. Together with the novel enzyme, this improves amplification efficiency of both GC- and AT-rich targets.
- 20 sec initial denaturation at 95 °C is sufficient for enzyme activation. When working with complex templates, an initial denaturation of 3 min is recommended.
- For two-step cycling, use 20 sec combined annealing/extension/data acquisition.
- Do not exceed 25 µl reaction volumes.

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

Quality Control

KAPA SYBR® FAST qPCR Master Mix (2X) is free of contaminating DNase and RNase. It is functionally tested by generating a standard curve with human genomic DNA as template, with a dynamic range of 5 orders of magnitude, a reaction efficiency of 90 - 110% and a correlation coefficient >0.99.

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, ABI 7300 Real-Time PCR Systems, GeneAmp® 5700, StepOne™, and the StepOnePlus™.

Step 1: Set up the qPCR reaction

- Ensure all reaction components are properly thawed and mixed.
- Calculate the required volumes of each component based on the following table:

Components	Final concentration	Per 20 µl rxn
PCR-grade water	-	Up to 20 µl
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)	As required

*Final $MgCl_2$ concentration is 2.5 mM.

- Prepare a PCR master mix consisting of the appropriate volumes of KAPA SYBR® FAST qPCR Master Mix (2X), PCR-grade water and any other component (e.g. template or primers) that is common to all or a subset of the reactions to be performed. Ensure reaction components are mixed properly

Step 2: Set up the plate

- Transfer the appropriate volume of reaction mixture to each well of a PCR tube/plate. Reaction volumes may be scaled down as required.
- Cap or seal the reaction tube/plate and centrifuge briefly.

Step 3: Run the qPCR Reaction

- If applicable, select fast mode on the instrument.
- Confirm that qPCR protocol to be used conforms to the following parameters:

Step	Temperature	Duration	Cycles
Enzyme activation	95 ºC	3 min*	Hold
Denature	95 ºC	1 - 3 sec	40
Anneal/ extend***	60 ºC	≥ 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 shortest time possible for instrument, but not less than 20 sec.

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

Note: The above cycling parameters are not optimal for qPCR-based quantification of next-generation sequencing libraries. Please refer to the protocol in the KAPA Library Quantification Kit Technical Data Sheet.

Step 4: Analyze the results

- Melt curve analysis should be performed to identify the presence of primer-dimers and analyze the specificity of the reaction. Program your thermocycler according to the instrument guidelines.
- Data analysis is dependent on experimental design. Refer to your instrument guidelines for more information on how to perform the appropriate data analysis.

Important Parameters

Template

High concentrations of template may increase background fluorescence and reduce linearity of standard curves. 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 proportionally). 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 (reverse transcription reaction product) 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).

Important Parameters (continued)

Primers

Careful primer design and purification (HPLC-purified primers are recommended) will minimize loss in sensitivity due to nonspecific amplification. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest primer concentration determined not to compromise reaction efficiency (50 - 400 nM of each primer). For optimal results, design primers that amplify PCR products 60 - 400 bp in length. Use appropriate primer design software to design primers with a melting temperature (T_m) of approximately 60 °C, to take advantage of two-step cycling. If performing qRT-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.

KAPA SYBR® DNA Polymerase

KAPA SYBR® DNA Polymerase is an engineered version of Taq DNA polymerase, designed specifically for real-time PCR using SYBR Green I chemistry. KAPA SYBR® DNA Polymerase displays no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer-dimers during reaction setup, prior to the first denaturation step, and results in high PCR specificity and accurate quantification. The enzyme is activated during the initial denaturation step of the PCR. 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 obviates the need to cool reactions during setup.

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 KAPA SYBR® DNA Polymerase. SYBR® Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding.

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.

Note to Purchaser: Limited License

Troubleshooting

Symptoms	Possible Causes	Solutions
Positive signal in no-template control (NTC) Presence of secondary, nonspecific peak in melt curve of sample	Reasons for a positive signal in a NTC and/or nonspecific amplifcation are often due to multiple factors that include: 1. Contamination 2. Primer-dimer formation due to: • Incorrect cycling protocol • Sub-optimal primer annealing temperature (often due to differences between qPCR systems) • Primer and/or template degradation (always store and dilute primers and template in 10 mM Tris-HCl, pH 8.0 - 8.5 and not in PCR-grade water) • Sub-optimal primer synthesis • Sub-optimal primer design	Perform melt curve analysis (or run qPCR products on a gel) to determine if the product is specific or nonspecific (primer dimer). If the NTC contains a specific product, the assay is contaminated: • Discard all reagents, clean all pipettes and surfaces and prepare fresh stocks of primer, etc. **Note:** The increased sensitivity of KAPA SYBR® FAST qPCR Kits may result in the detection of low levels of contamination in assays considered contaminant-free when using competitor kits containing wild-type Taq DNA polymerase. If the NTC and/or sample contains nonspecific product, assay optimization may be required: • 30 sec combined annealing/extension time is recommended for most assays. Longer times may result in nonspecific amplification. • Increase the combined annealing/extension temperature in increments of 3 °C. • Decrease primer concentration. • Resynthesize or redesign primers. HPLC purified primers are recommended for low copy number detection, and often results in reduced primer-dimer formation.
Low fluorescence intensity	Incorrect handling Incorrect concentration of ROX reference dye	SYBR® Green I dye is light sensitive; avoid exposure to light and repeated freeze-thaw cycles. Always thaw and mix solutions thoroughly before use. KAPA SYBR® FAST ABI Prism® qPCR Kits are recommended for the ABI 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™ instruments. The final concentration of ROX reference dye contained within the master mix is 500 nM. Analysis of the raw signal can always be performed with the ROX filter switched off when using ABI instrumentation.
Melting temperature of specific product is different from competitor kit	Differences in the buffer composition (e.g. salt concentration) of qPCR master mixes	Differences in master mix formulation may effect the melting temperature of the product slightly. A particular DNA fragment will melt at a higher temperature in a reaction buffer containing a higher salt concentration.

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