KAPABIOSYSTEMS

Technical Data Sheet

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

KR0389_S – v2.17

Product Description

KAPA SYBR FAST qPCR Master Mix (2X) is designed for high-performance real-time PCR. The kit contains a novel DNA polymerase—engineered via a process of molecular evolution—resulting in a unique enzyme specifically designed for real-time quantitative PCR (qPCR) using SYBR Green I dye chemistry.

KAPA SYBR FAST DNA Polymerase has been engineered to perform optimally in stringent qPCR reaction conditions, exhibiting dramatic improvements in signal-to-noise ratio (fluorescence), quantification cycle (Cq), linearity, and sensitivity. The KAPA SYBR FAST 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) Kits are 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, $MgCl_2$, dNTPs, and stabilizers.

Ensure that the correct KAPA SYBR FAST qPCR Master Mix (2X) is used in accordance with the reference dye requirements (if any) of the qPCR instrument (Table 1).

Product Applications

KAPA SYBR FAST qPCR Kits are ideally suited for:

- gene expression analysis;
- gene knockdown validation;
- microarray validation;
- low copy gene detection; and
- absolute quantification of NGS libraries (when sold as part of the KAPA Library Quantification Kit).

Kit Codes and Components	
KAPA SYBR FAST qPCR Master Mix (2X) Universal qPCR Master Mix (2X) ROX High Reference Dye (50X) ROX Low Reference Dye (50X)	**KK4600** (1 mL; 100 x 20 µL rxn)
	KK4601 (5 mL; 500 x 20 µL rxn)
	KK4602 (10 mL; 1000 x 20 µL rxn)
	KK4618 (50 mL; 5000 x 20 µL rxn)
KAPA SYBR FAST qPCR Master Mix (2X) ABI Prism™ qPCR Master Mix (2X) with ROX High incorporated in the reaction mix	**KK4603** (1 mL; 100 x 20 µL rxn)
	KK4604 (5 mL; 500 x 20 µL rxn)
	KK4605 (10 mL; 1000 x 20 µL rxn)
	KK4617 (50 mL; 5000 x 20 µL rxn)
KAPA SYBR FAST qPCR Master Mix (2X) optimized for LightCycler® 480 qPCR Master Mix (2X) with no passive reference dye	**KK4609** (1 mL; 100 x 20 µL rxn)
	KK4610 (5 mL; 500 x 20 µL rxn)
	KK4611 (10 mL; 1000 x 20 µL rxn)
KAPA SYBR FAST qPCR Master Mix (2X) ROX Low qPCR Master Mix (2X) with ROX Low incorporated in the reaction mix	**KK4619** (1 mL; 100 x 20 µL rxn)
	KK4620 (5 mL; 500 x 20 µL rxn)
	KK4621 (10 mL; 1000 x 20 µL rxn)
	KK4622 (50 mL; 5000 x 20 µL rxn)
KAPA SYBR FAST qPCR Master Mix (2X) Bio-Rad iCycler qPCR Master Mix (2X) with fluorescein incorporated in the reaction mix	**KK4606** (1 mL; 100 x 20 µL rxn)
	KK4607 (5 mL; 500 x 20 µL rxn)
	KK4608 (10 mL; 1000 x 20 µL rxn)

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 3-step cycling, use 20 sec for primer annealing and 1 sec for extension/data acquisition at 72°C.
- Do not exceed 25 µL reaction volumes.

Product Specifications

Shipping and Storage

KAPA SYBR FAST qPCR Master Mix (2X) Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store all components at -15°C to -25°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. KAPA SYBR FAST qPCR Master Mix (2X) may not freeze solidly, even when stored at -15°C to -25°C.

The SYBR Green I dye contained in KAPA SYBR FAST qPCR Master Mix (2X) and ROX/fluorescein dyes (depending on kit configuration) are light sensitive. Exposure to direct light for an extended period of time will result in loss of fluorescent signal intensity.

KAPA SYBR FAST qPCR Master Mix (2X) is stable through 30 freeze-thaw cycles. Ensure that all reagents are stored protected from light at -15°C to -25°C when not in use. When protected from light, reagents are stable in the dark at 2°C to 8°C for at least one week and may be stored at this temperature for short-term use, provided that they do not become contaminated with microbes and/or nucleases.

Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination. Please contact Technical Support at **sigma-aldrich.com/techservice** for more information.

Table 1. Instrument Compatibility*

Instrument	Reference Dye
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High 500 nM
Applied Biosystems 7500, ViiA™7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™ and Mx4000™	ROX Low 50 nM
Rotor-Gene™ instruments, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycler® 480, 96, Nano, 1.5/2.0**, Bio-Rad CFX96, Illumina® Eco™	No ROX
Bio-Rad iCyclers	Fluorescein

*For instruments not listed here, please contact Technical Support at **sigma-aldrich.com/techservice** for more information.

**The Roche LightCycler 1.5/2.0 capillary instruments require the addition of unacetylated BSA to the qPCR reaction at a final concentration of 250 ng/µL in order to prevent the DNA polymerase and template from binding to the glass capillaries.

Important Parameters

Template

High concentrations of template may increase background fluorescence and reduce linearity of standard curves. For optimal quantitative results, use ≤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 ≤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 ≤2.0 µL of undiluted cDNA).

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 (Tm) of approximately 60°C to take advantage of two-step cycling. If performing qRT-PCR, the design of primers specifically for amplification of cDNA derived from mRNA is recommended. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

KAPA SYBR FAST DNA Polymerase

KAPA SYBR FAST DNA Polymerase is an engineered version of *Taq* DNA polymerase, designed specifically for real-time PCR using SYBR Green I chemistry. KAPA SYBR FAST 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.

ROX Reference Dye

For certain real-time cyclers, the presence of ROX reference dye compensates for non-PCR-related variations in fluorescence detection. The fluorescence level of ROX reference dye does not change significantly 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 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.

Fluorescein Reference Dye

The use of fluorescein reference dye is necessary for Bio-Rad iCycler iQ®, iQ™5, and MyiQ™ instruments and is included in KAPA SYBR FAST qPCR Master Mix (2X) Bio-Rad iCycler at a final concentration of 10 nM. The presence of the fluorescein dye in this master mix does not interfere with real-time PCR on any instrument, since the dye is not involved in the reaction.

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

Magnesium Chloride

KAPA SYBR FAST qPCR Master Mix (2X) contains an optimized $MgCl_2$ concentration. It is highly unlikely that additional $MgCl_2$ will improve reaction efficiency or specificity.

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.

1. Master Mix Preparation

1.1 Ensure all reaction components are properly thawed and mixed.

1.2 Prepare a master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.

1.3 Always include a No Template Control (NTC) to allow for detection of contamination of reaction components.

1.4 Calculate the required volume of each component based on the following tables:

For Universal qPCR master mix (20 µL rxn[1])

Component	ROX	No ROX	Final conc.
PCR-grade water	Up to 20 µL	Up to 20 µL	N/A
KAPA SYBR FAST qPCR Master Mix (2X) Universal[2]	10 µL	10 µL	1X
10 µM forward primer	0.4 µL	0.4 µL	200 nM
10 µM reverse primer	0.4 µL	0.4 µL	200 nM
Template DNA[3]	As required	As required	<20 ng
50X ROX High/Low (as required)[4]	0.4 µL	—	1X

[1]Reaction volumes may be adjusted from 3 – 25 µL, depending on the block type and instrument used. Reaction volumes >25 µL are not recommended.

[2]KAPA SYBR FAST qPCR Master Mix contains $MgCl_2$ at a final concentration of 2.5 mM.

[3]Do not exceed 20 ng per 20 µL reaction. For more information, refer to **Important Parameters: Template**.

4The use of ROX dye is necessary for all Applied Biosystems® instruments and is optional for the Agilent Mx3000P™, Mx3005P™, and Mx4000™ cyclers. Bio-Rad/MJ Research, Cepheid, Corbett/ QIAGEN, Eppendorf, Illumina®, and Roche instruments do not require ROX dye.

For ABI Prism™, Bio-Rad iCycler™, LightCycler® 480 or ROX Low qPCR master mix (20 µL rxn[1])

Component	Volume	Final conc.
PCR-grade water	Up to 20 µL	N/A
KAPA SYBR FAST qPCR Master Mix (2X)[2]	10 µL	1X
10 µM forward primer	0.4 µL	200 nM
10 µM reverse primer	0.4 µL	200 nM
Template DNA[3]	As required	<20 ng

[1]Reaction volumes may be adjusted from 3 – 25 µL, depending on the block type and instrument used. Reaction volumes >25 µL are not recommended.

[2]KAPA SYBR FAST qPCR Master Mix (2X) contains $MgCl_2$ at a final concentration of 2.5 mM.

[3]Do not exceed 20 ng per 20 µL reaction. For more information, refer to **Important Parameters: Template**.

2. Reaction Setup

2.1 Transfer the appropriate volumes of qPCR master mix, template, and primers to each well of a PCR plate/tube(s).

2.2 Cap or seal the reaction plate/tube(s) and centrifuge briefly.

3. qPCR

3.1 If applicable, select fast mode on the instrument.

3.2 Confirm that the qPCR protocol to be used conforms to the following parameters:

For all cyclers *except* Roche LightCycler

Step	Temp.	Duration	Cycles
Enzyme activation	95°C	3 min[1]	Hold
Denaturation	95°C	1 – 3 sec	40
Annealing/ extension/ data acquisition[2]	60°C	≥20 sec[3]	
Dissociation	According to instrument guidelines		

[1]20 sec at 95°C is sufficient time for enzyme activation; however, optimal denaturation of complex targets may require up to 3 min denaturation.

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

[3]Select shortest time possible for instrument, but not <20 sec.

For Roche LightCycler®

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 acq/°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 denaturation.

[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 <20 sec for primer annealing.

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

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**.

4. Data Analysis

4.1 Data analysis is dependent on experimental design. Refer to the instrument guidelines for more information on how to perform the appropriate data analysis.

Troubleshooting

Symptoms	Possible Causes	Solutions
Positive signal in no-template control (NTC)	Contamination with specific product	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: • 20 – 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 3ºC • Decrease primer concentration.
Presence of secondary, nonspecific peak in melt curve of sample	Primer-dimer formation	Resynthesize or redesign primers. HPLC purification of primers greatly reduces dimer formation and increases sensitivity. Adjust primer concentration and annealing temperature to prevent dimer formation.
Low fluorescence intensity	Incorrect handling	SYBR Green I dye is light sensitive; avoid exposure to light and repeated freeze-thaw cycles. Always thaw and mix solutions thoroughly before use.
	Incorrect ROX concentration	If the incorrect concentration of ROX reference dye is added to the master mix, the normalized signal may be lower than expected (if too much ROX has been added), or higher than expected (if too little ROX has been added). If using ABI instrumentation, analysis of the raw signal can always be performed with the ROX filter switched off.
No product detected during qPCR, melting curve analysis or agarose gel electrophoresis	Template DNA contains inhibitors, or is degraded	Re-purify or re-isolate template DNA. Run DNA with a 1:10 dilution to dilute out inhibitors.
	Incorrect primer design or annealing temperature	Verify primer design. Lower annealing temperature in 2°C increments.
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 affect 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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