KAPABIOSYSTEMS

Technical Data Sheet

KAPA SYBR® FAST One-Step qRT-PCR Master Mix (2X) Kit

KR0393_S – v4.25

Product Description

The KAPA SYBR FAST One-Step qRT-PCR Master Mix (2X) Kit is a sensitive and convenient solution for real-time PCR using RNA as template. All kits contain, at minimum, KAPA SYBR FAST Master Mix (2X), KAPA RT Mix (50X), and dUTP (10 mM). The KAPA RT Mix comprises wild-type M-MuLV Reverse Transcriptase and RNase Inhibitor and is optimized for rapid one-step, one-tube RNA quantification.

KAPA SYBR FAST qPCR Master Mix (2X) contains a novel DNA polymerase engineered via a process of directed evolution. The result is a unique enzyme, specifically designed for high-performance real-time PCR using SYBR Green I dye chemistry. The 2X master mix is a ready-to-use cocktail containing all components for the amplification and detection of cDNA, except primers and template.

The use of dUTP at the recommended final concentration results in amplicons that can be degraded using Uracil-DNA Glycosylase (UDG). UDG treatment is performed in subsequent reactions in order to minimize carryover PCR contamination downstream. Use of dUTP in this system is optional—UDG is not supplied in the kit.

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 One-Step qRT-PCR Master Mix (2X) Kits are ideally suited for:

- gene expression analysis;
- gene knockdown validation;
- microarray validation;
- low copy gene detection; and
- RNAi and miRNA research.

Kit Codes and Components	
KAPA SYBR FAST One-Step qRT-PCR Master Mix (2X) Universal	**KK4650** (100 x 20 µL reactions)
qPCR Master Mix (2X) ROX High Reference Dye (50X) ROX Low Reference Dye (50X) dUTP (10 mM) KAPA RT Mix (50X)	**KK4651** (500 x 20 µL reactions)
	KK4652 (1000 x 20 µL reactions)
KAPA SYBR FAST One-Step qRT-PCR Master Mix (2X) Bio-Rad iCycler	**KK4670** (100 x 20 µL reactions)
qPCR Master Mix (2X) with fluorescein incorporated in the reaction mix dUTP (10 mM) KAPA RT Mix (50X)	**KK4671** (500 x 20 µL reactions)
	KK4672 (1000 x 20 µL reactions)

Quick Notes

- This kit contains wild-type M-MuLV and 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.
- Use only gene-specific primers for one-step qRT-PCR.
- Optimal cDNA synthesis is achieved at 42°C for 5 min.
- 3 min at 95°C is sufficient for RT inactivation and DNA polymerase activation.
- 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 qRT-PCR 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. The KAPA SYBR FAST qPCR Master Mix (2X) may not freeze solidly, even when stored at -15°C to -25°C. The KAPA RT Mix is temperature-sensitive, and should be stored at -15°C to -25°C and kept on ice during use.

The SYBR Green I dye contained in the 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.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at www.sigmaaldrich.com, or upon request from www.sigma-aldrich.com/techservice.

Important Parameters

Template RNA

Starting template of purified total RNA can range between 1 pg – 100 ng per 20 µL reaction. Using greater amounts of template may increase the background fluorescence, which reduces linearity of standard curves after background subtraction. Digest purified RNA with RNase-free DNase I to remove contaminating genomic DNA which can act as template during PCR. DNase treatment should be performed according to manufacturer's instructions. Treated RNA should be stored at -20°C or -80°C in RNase-free water. Multiple freeze-thaws of RNA should be avoided.

Primers

Careful primer design and purification (HPLC-purified primers are recommended) will minimize loss in sensitivity due to non-specific amplification. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest primer concentration that does not compromise reaction efficiency (50 – 400 nM of each primer). For optimal results, design primers that amplify PCR products 70 – 200 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. Primers must be carefully designed to avoid detection and amplification of genomic DNA, which would lead to inaccurate mRNA quantification. To prevent gDNA amplification, design forward and reverse primers from different exons, or to span exon-intron boundaries.

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.

KAPA RT Mix

KAPA RT Mix (50X) contains an optimized blend of wild-type M-MuLV Reverse Transcriptase and RNase Inhibitor. M-MuLV RT has a high affinity for RNA and is optimized for cDNA synthesis at 42°C. The RNase Inhibitor safeguards against degradation of RNA target due to RNase contamination. KAPA RT Mix (50X) **must** be stored at -15°C to -25°C as the enzymes are not thermostable, and must be worked with on ice at all times.

dUTP

Use of dUTP allows treatment with uracil-DNA glycosylase (UDG) if required to prevent carryover contamination in subsequent reactions. dUTP can be used at final concentrations that range from 0.2 – 0.4 mM. ***Do not use UDG in one-step qRT-PCR***, as the UDG will degrade the cDNA upon synthesis.

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 the KAPA SYBR FAST One-Step qRT-PCR Master Mix (2X) for 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.

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.

Protocol

Any existing one-step qRT-PCR assay performed efficiently using standard cycling conditions may be converted to a fast, one-step qRT-PCR assay with KAPA SYBR FAST One-Step qRT-PCR 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 Keep the KAPA RT Mix and assembled reactions on ice during use, to avoid premature cDNA synthesis.

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

1.4 Include a No Template Control (NTC) and No RT Control (NRT). The NTC will enable detection of contamination of reaction components, while the NRT will enable detection of contaminating gDNA.

1.5 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 mM dUTP (optional)	0.4 µL	0.4 µL	0.2 mM
10 µM forward primer	0.4 µL	0.4 µL	200 nM
10 µM reverse primer	0.4 µL	0.4 µL	200 nM
50X KAPA RT Mix	0.4 µL	0.4 µL	1X
Template RNA[3]	As required	As required	1 pg – 100 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]Template RNA input of 1 pg – 100 ng total RNA is recommended. For more information, refer to **Important Parameters: Template**.

[4]The 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 Bio-Rad iCycler™, (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 mM dUTP (optional)	0.4 µL	0.2 mM
10 µM forward primer	0.4 µL	200 nM
10 µM reverse primer	0.4 µL	200 nM
50X KAPA RT Mix	0.4 µL	1X
Template RNA[3]	As required	1 pg – 100 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]Template RNA input of 1 pg – 100 ng total RNA is recommended. 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. One-step 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
Reverse transcription[1]	42°C	5 min	Hold
Enzyme activation	95°C	3 min	Hold
Denaturation	95°C	1 – 3 sec	40
Annealing/ extension/ data acquisition[2]	60°C	≥20 sec[3]	
Dissociation	According to instrument guidelines		

[1]5 min at 42°C is sufficient for cDNA synthesis. For difficult assays, this may be increased to 10 min.

[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**	
Reverse transcription	1	None	
Amplification	40[1]	Quantification	
Melting curve	1	Melting curves	
Cooling	1	None	
Program Name	**Target (°C)**	**Acquisition Mode**	**Hold (hh:mm:ss)**
Reverse transcription	42	None	00:05:00[2]
	95	None	00:03:00
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]5 min at 42°C is sufficient for cDNA synthesis. For difficult assays, this may be increased to 10 min.

[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.

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) or no-RT control (NRT)	RNA template contaminated with genomic DNA	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: • Take standard precautions to avoid contamination during reaction setup. Treat RNA sample with RNase-free DNase I. **Note:** the increased sensitivity of KAPA SYBR FAST One-Step qRT-PCR 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	Incorrect cycling protocol was used	Ensure that the cycling protocol contains the cDNA synthesis step, and that the correct fluorescent detection channel is selected.
	Pipetting error or missing reagent	Ensure that correct reagents have been used.
	Template RNA contains inhibitors, or is degraded	Re-purify or re-isolate template RNA.
	Incorrect primer design or annealing temperature	Verify primer design. Lower annealing temperature in 2°C increments.
Product detected later than expected	Amplicon is too long	Optimal results are obtained with amplicons of 70 – 200 bp.
	PCR annealing/extension time is too short	This kit requires a minimum of 20 sec annealing followed by 1 sec extension at 72°C for optimal performance.
	Template contains inhibitors or is degraded	Re-purify or re-isolate template RNA.
Poor low copy number sensitivity	Sub-optimal primer design or annealing temperature	Redesign primers. HPLC purification of primers greatly reduces primer-dimer problems and increases sensitivity. Adjust primer concentration and T_m. Ensure that the correct cycling parameters were used.
High baseline fluorescence	Starting amount of template is too high	Reduce the amount of template in the reaction.
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.

KAPABIOSYSTEMS

Manufacturing, R&D
Cape Town, South Africa
Tel: +27 21 448 8200

Technical Support
sigma-aldrich.com/techservice