



KAPA™ SYBR® FAST One-Step qRT-PCR Kit Universal

1. Product Description

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

The KAPA SYBR® FAST Master Mix (2X) contains a novel DNA polymerase engineered via molecular evolution and optimized for qPCR using SYBR® Green I dye chemistry. The 2X Master Mix is a ready-to-use cocktail containing all components except primers and template for the amplification and detection of cDNA. ROX reference dye is not included in the 2X Master Mix but is supplied separately.

dUTP (10 mM) is also supplied in the kit. 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 carry over PCR contamination downstream. Use of dUTP in this system is optional. UDG is not supplied in this kit.

2. Product Applications

KAPA SYBR® FAST One-Step qRT-PCR Kits are ideally suited for:

- Gene expression analysis
- Low copy gene detection
- Microarray validation
- Gene knockdown validation
- RNAi and miRNA research

3. Product Specifications

3.1 Storage and handling

Upon arrival, store kit components away from light and 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 18 months from the date of receipt. The KAPA RT Mix (50X) is guaranteed for 6 months at -20 °C.

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.

3.2 Quality Control

Kit components are free of contaminating DNase and RNase. They are functionally tested to demonstrate a resolution of five orders of linear dynamic range.

3.3 Product Use Limitations

Kit components are sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, were 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.

KK4650

100 reactions in 20 µl volume

KAPA SYBR® FAST One-Step qRT-PCR Kit Universal 1 x 1 ml

- Contains:
- KAPA SYBR® FAST qPCR Master Mix (2X)
 - ROX Reference Dye High (50X)* 1 x 200 µl
 - ROX Reference Dye Low (50X)* 1 x 200 µl
 - dUTP (10 mM) 1 x 40 µl
 - KAPA RT Mix (50X) 1 x 40 µl

KK4651

500 reactions in 20 µl volume

KAPA SYBR® FAST One-Step qRT-PCR Kit Universal 1 x 5 ml

- Contains:
- KAPA SYBR® FAST qPCR Master Mix (2X)
 - ROX Reference Dye High (50X)* 1 x 200 µl
 - ROX Reference Dye Low (50X)* 1 x 200 µl
 - dUTP (10 mM) 1 x 200 µl
 - KAPA RT Mix (50X) 1 x 200 µl

KK4652

1000 reactions in 20 µl volume

KAPA SYBR® FAST One-Step qRT-PCR Kit Universal 2 x 5 ml

- Contains:
- KAPA SYBR® FAST qPCR Master Mix (2X)
 - ROX Reference Dye High (50X)* 2 x 200 µl
 - ROX Reference Dye Low (50X)* 2 x 200 µl
 - dUTP (10 mM) 2 x 200 µl
 - KAPA RT Mix (50X) 2 x 200 µl

*Please see Section 5.7 for details on ROX reference dye concentrations
The final MgCl₂ concentration per reaction is 2.5 mM

Quick Notes

- This kit contains wild-type M-MuLV and an engineered DNA polymerase optimized for use in one-step qRT-PCR using SYBR® Green I dye.
- The 2X Master Mix contains a proprietary buffer that, together with the novel polymerase, enhances the amplification efficiency of both high GC and high AT templates.
- Optimal cDNA synthesis is achieved at 42 °C for 5 min.
- Use only gene specific primers for one-step qRT-PCR.
- KAPA RT Mix (50X) must be stored at -20 °C as the enzyme is not thermostable.
- 2 - 5 min at 95 °C is sufficient for RT inactivation and DNA polymerase activation.
- For two-step cycling, use ≥20 sec combined annealing/extension and data acquisition.
- Do not exceed 25 µl reaction volumes.



4. KAPA SYBR® FAST One-Step qRT-PCR 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.

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, StepOne™, StepOnePlus™, ViiA™7, the Stratagene Mx3000P®, Mx3005P™, and Mx4000®, the Qiagen/Corbett Research Rotor-Gene™, the MJ Research DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector, Eppendorf Mastercycler® ep realplex, Roche LightCycler® 480, Bio-Rad CFX96, Illumina Eco Real-Time PCR System, and the Cepheid Smart Cycler®.

4.1 Step 1: qPCR Reaction Setup

- Before preparing qRT-PCR reactions, thoroughly mix the KAPA SYBR® FAST qPCR Master Mix (2X), KAPA RT Mix (50X), dUTP (10 mM), ROX Reference Dye High/Low, template RNA, and primers.
- Keep all kit components and assemble all reactions on ice to avoid premature cDNA synthesis.
- The recommended RNA input is from 1 pg to 100 ng total RNA.
- Prepare a reaction cocktail to reduce pipetting errors. Dispense equal aliquots into reaction tubes. Add RNA to each reaction as a final step. Addition of 2 - 5 µl volumes of RNA will improve assay precision.
- Include a No Template Control (NTC) and No RT Control (NRT) when necessary. The NTC will enable detection of contamination in the reaction components. The NRT control tests for contaminating genomic DNA in the reaction.
- Calculate the required volumes of each component based on the following table:

	Final concentration	20 µl rxn
Nuclease-free 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
dUTP (10 mM) (optional)	200 µM	0.4 µl
ROX High/Low (optional)	See section 5.7	0.4 µl
KAPA RT Mix (50X)	1X	0.4 µl
Template RNA	variable	< 5 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
cDNA synthesis	42 °C	5 min	Hold
Inactivate RT	95 °C	2 - 5 min*	Hold
Denature	95 °C	3 sec	40
Anneal/extend***	60 °C	≥ 20 sec**	
Dissociation	According to instrument guidelines		

*20 sec at 95 °C is sufficient time for DNA polymerase activation, however 2 - 5 min is required to fully inactivate the reverse transcriptase prior to cycling.

**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 the minimum time required for data acquisition at 72 °C according to instrument user guide.



5. Important Parameters

5.1 Template

Starting template of purified total RNA can range between 1 pg to 100 ng per 20 µl reaction (for smaller reaction volumes, the amount of template should be decreased proportionally). Using greater amounts of template may increase the baseline fluorescence and hence reduce the maximum fluorescence signal and 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 manufacturers instructions. Treated RNA should be stored at -20 °C or -80 °C in RNase-free water. Multiple freeze-thawing of RNA should be avoided.

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 supports amplification without compromising the efficiency of the PCR reaction (50 - 400 nM of each primer). For optimal results, design primers that amplify PCR products 70 - 200 bp in length. The primers should exhibit a melting temperature (T_m) of approximately 60 °C. qRT-PCR primers need to be carefully designed to avoid detection and amplification of genomic DNA, which would lead to inaccurate mRNA quantification. To minimize the effect of DNA contamination in RNA template, the forward and reverse primers can be designed from different exons and to span exon-intron boundaries. In this way one can reduce the genomic DNA contribution to expression quantification.

5.3 KAPA RT Mix (50X)

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 -20 °C as the enzymes are not thermostable.

5.4 KAPA SYBR® DNA Polymerase

KAPA SYBR® DNA polymerase is an engineered version of Taq DNA polymerase optimized for real-time PCR. It is supplied in a hot start formulation and is inactive at ambient temperature. Activation of this enzyme is complete after 20 sec at 95 °C, however 2 - 5 min is required to completely deactivate the reverse transcriptase in the same step.

5.5 dUTP (10 mM)

Use of dUTP allows optional treatment with Uracil-DNA Glycosylase (UDG) if prevention of carry over contamination in subsequent reactions is required. dUTP can be used at final concentrations that range from 0.2 - 0.4 mM. Do not use UDG in a one-step qRT-PCR reaction as the UDG will degrade the cDNA upon synthesis.

5.6 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.

5.7 ROX Reference Dye

For certain real-time PCR 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, Qiagen/Corbett Research, Eppendorf, Illumina, 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™, StepOnePlus™, and ViiA™ 7	0.4 µl*	-
ABI 7500 Stratagene Mx3000P®, Mx3005P™, and Mx4000®	-	0.4 µl**

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



6. Troubleshooting

Symptom	Possible Cause	Solution
High baseline fluorescence No product on either qPCR graph or on an agarose gel	Starting amount of template is too high The protocol was not followed correctly Pipetting error or missing reagent Template contains inhibitors or is degraded Incorrect primer design or annealing temperature is too high	Reduce the amount of template in the reaction. Ensure the cycling program includes the cDNA synthesis step and the correct detection channel has been selected. Ensure that the correct reagents and concentrations have been used. Re-purify or re-isolate your template. Verify primer selection. Lower the annealing temperature in 2 °C decrements.
Product detected later than expected	Amplicon length is too long PCR annealing/extension time is too short for optimal performance. MgCl ₂ concentration adjusted Template contains inhibitors or is degraded	Optimal results are obtained with amplicons of 70 - 200 bp. Verify that the correct cycling protocol has been used. Do not adjust the MgCl ₂ concentration of KAPA SYBR® qPCR Master Mix (2X). Re-purify or re-isolate RNA or dilute RNA in 10 mM Tris pH 8.5.
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 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 NTC or NRT control	RNA template contaminated with genomic DNA Primer-dimer formation	Take standard precautions to avoid contamination when preparing your PCR reactions. Treat RNA sample with RNase-free DNase I. 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).

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For technical support please contact:
support@kapabiosystems.com

Boston, Massachusetts, United States

600 West Cummings Park, Suite 2250
Woburn, MA, 01801 U.S.A.
Tel: +1 781 497 2933 Fax: +1 781 497 2934
Email: info@kapabiosystems.com

Cape Town, South Africa

2nd Floor, Old Warehouse Building, Black River Park,
Fir Road, Observatory, 7925 Cape Town, South Africa
Tel: +27 21 448 8200 Fax: +27 21 448 6503
Email: info@kapabiosystems.com