KAPABIOSYSTEMS

Technical Data Sheet

KAPA PROBE FAST qPCR Master Mix (2X) Kit

KR0397_S – v1.17

Product Description

KAPA PROBE FAST qPCR Master Mix (2X) Kits are designed for fast-cycling, real-time PCR (qPCR) using sequence-specific fluorogenic probes. These kits are compatible with all fluorogenic probe-based technologies, including hybridization probes (e.g., FRET), hydrolysis probes (e.g., TaqMan®) and displacement probes (e.g., molecular beacons).

KAPA PROBE FAST qPCR Master Mix (2X) Kits contain a ready-to-use cocktail containing all components except primers, probe(s), and template for fast-cycling probe-based qPCR. The 2X master mix contains KAPA Taq HotStart DNA Polymerase, dNTPs, $MgCl_2$, and stabilizers.

KAPA Taq HotStart DNA Polymerase is an antibody-mediated hotstart formulation of KAPA Taq DNA Polymerase. In the HotStart formulation, the enzyme is combined with a proprietary antibody that inactivates the enzyme until the first denaturation step. This eliminates spurious amplification products resulting from non-specific priming events during reaction setup and initiation, and increases overall reaction efficiency.

Ensure that the correct KAPA PROBE 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 PROBE FAST qPCR Kits are ideally suited for:

- gene expression analysis;
- SNP genotyping/allelic discrimination; and
- microarray validation.

Kit Codes and Components	
KAPA PROBE FAST qPCR Master Mix (2X) Universal qPCR Master Mix (2X) ROX High Reference Dye (50X) ROX Low Reference Dye (50X)	**KK4701** (100 x 20 µL reactions)
	KK4702 (500 x 20 µL reactions)
	KK4703 (1000 x 20 µL reactions)
	KK4715 (5000 x 20 µL reactions)
KAPA PROBE FAST qPCR Master Mix (2X) ABI Prism™ qPCR Master Mix (2X) with ROX High incorporated	**KK4705** (100 x 20 µL reactions)
	KK4706 (500 x 20 µL reactions)
	KK4707 (1000 x 20 µL reactions)
	KK4714 (5000 x 20 µL reactions)
KAPA PROBE FAST qPCR Master Mix (2X) ROX Low qPCR Master Mix (2X) with ROX Low incorporated	**KK4716** (100 x 20 µL reactions)
	KK4717 (500 x 20 µL reactions)
	KK4718 (1000 x 20 µL reactions)
KAPA PROBE FAST qPCR Master Mix (2X) Bio-Rad iCycler qPCR Master Mix (2X) with fluorescein incorporated	**KK4709** (100 x 20 µL reactions)
	KK4710 (500 x 20 µL reactions)
	KK4711 (1000 x 20 µL reactions)

Quick Notes

- These kits are compatible with all fluorogenic probe-based technologies, including hybridization probes (e.g., FRET), hydrolysis probes (e.g., TaqMan®) and displacement probes (e.g., molecular beacons).
- Initial denaturation for 20 sec at 95°C is sufficient for enzyme reactivation; however, optimal denaturation of complex targets may require up to 3 min denaturation.
- For 2-step cycling, use 30 sec combined annealing/extension/data acquisition.
- For 3-step cycling, use 20 sec for primer annealing and 1 sec for extension/data acquisition.
- Do not exceed 25 µL volumes.

Product Specifications

Shipping and Storage

KAPA PROBE FAST qPCR Master Mix (2X) Kits are shipped on ice packs. Upon arrival, store kit components protected from light at -20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the 2X master mix is retained until the expiry date indicated on the kit label.

Handling

ROX/fluorescein reference 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. Avoid repeated freezing and thawing. Always ensure that the product has been fully thawed and mixed before use.

KAPA PROBE FAST qPCR Master Mix (2X) is stable through 30 freeze-thaw cycles. Ensure that all reagents are stored protected from light at -20°C when not in use. When protected from light, reagents are stable in the dark at 4°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

KAPA PROBE FAST qPCR Master Mix (2X) is subjected to stringent quality control tests, is free of contaminating exo- and endonuclease activity, and meets strict requirements with respect to DNA contamination levels. 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

Assay Design

The use of previously-validated assays or dedicated qPCR design software such as Beacon Designer 7 (www.PremierBiosoft.com) is recommended when designing probe-based assays.

Lyophilized primers and probes should be resuspended in 10 mM Tris-HCl (pH 8.0 – 8.5). DNA kept frozen in a nuclease-free environment should be stable for years. Initial preparation of a 100 µM freezer stock (which should be thawed relatively infrequently) may be convenient.

Optimal primer concentration should be determined empirically. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the qPCR reaction. The optimal primer concentration range is 100 – 400 nM.

Optimal probe concentration should be determined empirically. The optimal probe concentration range has generally been found to be 100 – 500 nM.

ROX Reference Dye

For certain real-time cyclers, the presence of the passive reference dye, ROX, compensates for non-PCR related variations in fluorescence detection. Fluorescence from the 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, the 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 Reference Dye is necessary for all Applied Biosystems instruments and is optional for the Stratagene Mx3000P™, Mx3005P™, and Mx4000™ cyclers. Bio-Rad/MJ Research, Cepheid, Corbett Research/QIAGEN, Eppendorf, and Roche instruments do not require ROX. The presence of the ROX reference 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 the fluorophore with which the probe is labelled.

Fluorescein Reference Dye

For certain Bio-Rad real-time cyclers, the presence of the passive reference dye, fluorescein, compensates for non-PCR–related variations in fluorescence detection. Fluorescence from the fluorescein 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, the fluorescein dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or differences in well position. KAPA PROBE FAST qPCR Master Mix (2X) for Bio-Rad iCycler™ contains fluorescein reference dye at an optimal concentration for use on the Bio-Rad iCycler™ iQ®, iQ™5, and MyiQ™ real-time instruments.

Magnesium Chloride

The concentration of $MgCl_2$ affects the binding dynamics of primers and probes to template DNA. The higher the final $MgCl_2$ concentration in the PCR reaction, the greater the binding affinity of the primers and probe for target DNA. KAPA PROBE FAST qPCR Master Mix (2X) provides $MgCl_2$ at a final (1X) concentration of 5.0 mM. 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 PROBE FAST qPCR Master Mix (2X) 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 PCR master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.

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

Component	ROX	No ROX	Final conc. (20 µL reaction[1])
PCR-grade water	Up to 20 µL	Up to 20 µL	N/A
KAPA PROBE FAST qPCR Master Mix[2]	10 µL	10 µL	1X
10 µM forward primer	0.2 – 0.8 µL	0.2 – 0.8 µL	100 – 400 nM
10 µM reverse primer	0.2 – 0.8 µL	0.2 – 0.8 µL	100 – 400 nM
10 µM probe	0.2 – 1.0 µL	0.2 – 1.0 µL	100 – 500 nM
Template DNA	As required	As required	<250 ng
50X ROX High/Low (as required)[3]	0.4 µL	—	1X

[1]Reaction volumes may be adjusted from 5 – 25 µL, depending on the block type and instrument used. Reaction volumes >25 µL are not recommended.

[2]KAPA PROBE FAST qPCR Master Mix (2X) contains $MgCl_2$ at a final concentration of 5 mM.

[3]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.

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:

Step	Temp.	Duration	Cycles
Enzyme activation	95°C	3 min[1]	Hold
Denaturation	95°C	1 – 3 sec	40
Annealing/ extension/ data acquisition[2]	55 – 65°C[3]	≥20 sec[4]	

[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 1 sec extension and data acquisition at 72ºC according to instrument guidelines.

[3]Annealing temperature is dependent on the specific primer/probe combination.

[4]Use 20 sec at 60ºC as a starting point. The minimum programmable annealing/extension time will vary for different instruments.

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
Late Cq or no amplification during cycling	Incorrect cycling protocol	Verify that the correct default cycling conditions were used.
	Incorrect reaction setup	Verify that all the components have been added at the correct concentrations.
	Incorrect detection filter/channel	Check that the correct filters have been selected for data acquisition.
	Degraded template DNA, primers or probe	Always store and dilute primers and DNA in 10 mM Tris-HCl (pH 8.0 – 8.5), and not in PCR-grade water.
	Sub-optimal primer/probe design	The use of pre-validated assays or the design of assays using dedicated software is recommended.
	Non-specific products may be amplified (confirm with agarose gel electrophoresis)	Repeat setup on ice and run qPCR reaction immediately after setup. Increase annealing temperature in 2°C increments.
	Incorrect annealing temperature	Decrease annealing temperature in 2°C increments.
Positive signal in no-template control (NTC)	Contamination of reagents	Discard all reagents and repeat experiment with new components.
	Contamination during setup	Review setup procedure and ensure that aerosol-barrier pipette tips are used.
	Degradation of primers and probe	Always store and dilute primers and DNA in 10 mM Tris-HCl (pH 8.0 – 8.5) and not in PCR-grade water. Use new stocks of primers and probe or redesign the assay.
Extremely high ΔRn or Rn values	ROX/fluorescein (if required) was not selected as the passive reference dye at setup	Select ROX as the passive reference dye when setting up the plate.
	The incorrect concentration of ROX Reference Dye was used for the qPCR platform (if required)	Refer to Table 1 for the correct ROX concentration for the qPCR platform being used.
High variability across replicates	Insufficient mixing of reaction master mix	Mix the reaction by inverting the tube a few times, followed by brief centrifugation prior to aliquoting to the reaction plate.
	Evaporation	Ensure that the optical lids or sealing film is completely sealed before loading the qPCR instrument. This is particularly important on the edges of qPCR plates.

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