



KAPA™ PROBE FAST qPCR Kit

Master Mix (2X) Universal

1. Product Description

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

KAPA PROBE FAST Universal qPCR Master Mix (2X) is a ready-to-use cocktail containing all components except primers, probe(s) and template for fast cycling probe-based real-time PCR. The 2X Master Mix contains KAPATaq HotStart DNA polymerase, KAPA PROBE FAST qPCR Buffer, dNTPs, MgCl₂ and stabilizers. ROX reference dye is not included in the 2X Master Mix but is supplied separately.

KAPATaq HotStart DNA Polymerase is an antibody-mediated hot start formulation of KAPATaq 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.

2. Product Applications

KAPA PROBE FAST Universal qPCR Kits are ideally suited for:

- Gene expression analysis
- SNP genotyping
- Microarray validation
- Gene knockdown validation

3. Product Specifications

3.1 Shipping and Storage

KAPA PROBE FAST Universal qPCR 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, all kit components will retain full activity for 18 months from the date of receipt.

3.2 Handling

ROX Reference Dyes are sensitive to exposure to light. Avoid repeated freezing and thawing. Always ensure that the product has been fully thawed and mixed before use.

3.3 Quality Control

KAPA PROBE FAST Universal qPCR Master Mix (2X) is free of contaminating DNase and RNase. It is functionally tested to demonstrate resolution of 5 orders of linear dynamic range using human genomic DNA as template and an ApoB100 primer/probe assay.

3.4 Product Use Limitations

KAPA PROBE FAST Universal qPCR Master Mix (2X) is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual component, was 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, which is available upon request.

KK4701

100 x 20 µl
reactions

KAPA PROBE FAST qPCR Master Mix (2X) Universal 1 x 1 ml

Contains:
- qPCR Master Mix (2X)
- ROX Reference Dye High (50X)* 1 x 200 µl
- ROX Reference Dye Low (50X)* 1 x 200 µl

KK4702

500 x 20 µl
reactions

KAPA PROBE FAST qPCR Master Mix (2X) Universal 1 x 5 ml

Contains:
- qPCR Master Mix (2X)
- ROX Reference Dye High (50X)* 1 x 200 µl
- ROX Reference Dye Low (50X)* 1 x 200 µl

KK4703

1000 x 20 µl
reactions

KAPA PROBE FAST qPCR Master Mix (2X) Universal 2 x 5 ml

Contains:
- qPCR Master Mix (2X)
- ROX Reference Dye High (50X)* 2 x 200 µl
- ROX Reference Dye Low (50X)* 2 x 200 µl

* See Section 5.2 for details

The final MgCl₂ concentration per PCR reaction is 5 mM

Quick Notes

- This kit is designed for high-throughput, fast cycling, real-time PCR using sequence-specific fluorogenic probes.
- The kit is suitable for all fluorogenic probe-based technologies, including 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 two-step cycling, use 30 sec combined annealing/extension/data acquisition.
- For three-step cycling, use 20 sec for primer annealing and 1 sec for extension/data acquisition.
- Do not exceed 25 µl reaction volumes.

Instrument Table

Instrument	ROX Reference Dye
ABI 5700, 7000, 7300, 7700, 7900HT, StepOne™ and StepOnePlus™	ROX High
ABI 7500 Stratagene Mx3000P®, Mx3005P™ and Mx4000®	ROX Low
Rotor-Gene™, Rotor-Gene Q, DNA Engine Opticon™, Opticon® 2 and Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler® Roche LightCycler® 480, Bio-Rad CFX96, Helixix Pixo™	No ROX



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4. KAPA PROBE FAST qPCR Protocol

Any existing qPCR assay performed efficiently using standard cycling conditions may be converted to a Fast qPCR assay with KAPA PROBE FAST qPCR Kits. Typically, minimal re-optimization of reaction parameters is required.

This protocol is intended for use with, but not limited to: the ABI PRISM®7000, 7700 and 7900HT, the ABI 5700, ABI 7300 and 7500 Real-Time PCR Systems, the Stratagene Mx3000P®, Mx3005P™ and Mx4000®, the Corbett Research Rotor-Gene™, the Rotor-Gene Q, the MJ Research DNA Engine Opticon™, Opticon® 2 and Chromo 4™ Real-Time Detector, the Eppendorf Mastercycler® ep realplex, the Roche LightCycler® 480, the Bio-Rad CFX96 and the Cepheid Smart Cycler®.

4.1 Step 1: qPCR Reaction Setup

- Before preparing qPCR reactions, thoroughly mix the KAPA PROBE FAST qPCR Master Mix (2X), ROX Reference Dye High/Low (see Section 5.2), template DNA, primers and probes.
- Calculate the required volumes of each component based on the following table:

	Final concentration	20 µl rxn
PCR grade water up to 20 µl		As required
KAPA PROBE FAST qPCR Master Mix (2X) - Universal	1X	10 µl
Forward Primer (10 µM)	100 - 400 nM	Variable
Reverse Primer (10 µM)	100 - 400 nM	Variable
Probe	100 - 500 nM	Variable
Template DNA or cDNA	<250 ng	Variable

4.2 Step 2: Plate Setup

- Preparation of a reaction cocktail is vital in qPCR to reduce the effect of pipetting errors between samples. Assemble all components above except template DNA or cDNA.
- Gently mix all components in the cocktail before transferring the appropriate volume of reaction mixture to each well of a PCR tube/plate.
- Add template DNA or cDNA to each reaction.
- 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
Enzyme activation	95 °C	20 sec - 3 min ¹	Hold
Denature	95 °C	1 - 3 sec	40
Anneal/Extend/Acquire ²	55 - 65 °C ³	15 - 30 sec ⁴	

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

²For 3-step cycling protocols, anneal at optimal annealing temperature for 20 sec, followed by 1 sec extension and data acquisition at 72 °C.

³Dependant on the specific primer/probe combination.

⁴Use 20 sec at 60 °C as a general starting point. The minimum programmable extension time will vary for different instrument platforms.

4.4 Step 4: Analyze the results

- Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.



5. Important Parameters

5.1 Assay Design

We recommend using previously validated assays or using dedicated qPCR design software such as Beacon Designer 7 when designing Probe-based assays (www.PremierBiosoft.com).

Lyophilized primers and probes should be resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. DNA kept frozen in a nuclease-free environment should be stable for years. We find it convenient to initially prepare a 100 μ M freezer stock (which should be thawed relatively infrequently).

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.

5.2 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, 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.

Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 20 μ l reaction	
	ROX High (50X)	ROX Low (50X)
ABI 5700, 7000, 7300, 7000, 7900HT StepOne [™] and StepOnePlus [™]	0.4 μ l*	-
ABI 7500 Stratagene Mx3000P [®] , Mx3005P [™] and Mx4000 [®]	-	0.4 μ l**

*Final concentration = 500 nM **Final concentration = 50 nM

5.3 MgCl₂

The concentration of MgCl₂ affects the binding dynamics of primers and probes to template DNA. The higher the final MgCl₂ concentration in the PCR reaction, the greater the binding affinity of the primers and probe for target DNA. KAPA PROBE FAST Universal qPCR Master Mix (2X) provides MgCl₂ at a final concentration of 5 mM, which is suitable for most targets.



6. Troubleshooting

Symptom	Possible Cause	Solution
Late C _t or no amplification during cycling	<p>Incorrect cycling protocol</p> <p>Incorrect reaction setup</p> <p>Incorrect detection filter/channel</p> <p>Degraded template DNA</p> <p>Sub-optimal primer/probe design</p> <p>Degraded probe or primers</p> <p>Non-specific products may be amplified</p> <p>Incorrect annealing temperature</p>	<p>Verify that the correct default cycling conditions were used.</p> <p>Verify that all the components have been added at the correct concentrations.</p> <p>Check that the correct filters have been selected for data acquisition.</p> <p>Prepare fresh template then repeat experiment.</p> <p>We recommend using pre-validated assays or designing them using dedicated software.</p> <p>Check integrity of primers/probe on a denaturing polyacrylamide gel.</p> <p>Repeat setup on ice and run qPCR reaction immediately after setup.</p> <p>Increase annealing temperature in 2 °C increments.</p> <p>Decrease annealing temperature in 2 °C increments.</p>
The NTC gives a positive result	<p>Contamination of reagents</p> <p>Contamination during setup</p> <p>Degradation of primers and probe</p>	<p>Discard all reagents and repeat experiment with new components.</p> <p>Review setup procedure and ensure that aerosol-barrier pipette tips are used.</p> <p>Use new stocks of primers and probe or redesign the assay.</p>
Extremely high ΔRn or Rn values	<p>ROX was not selected as the passive reference dye at setup, or the incorrect concentration of ROX Reference Dye was used for the qPCR platform</p>	<p>Select ROX as the passive reference dye when setting up the plate.</p> <p>Refer to Section 5.2 for details on correct ROX concentration for qPCR platform.</p>
High variability across replicates	<p>Insufficient mixing of reaction master mix</p> <p>Evaporation</p>	<p>Mix the reaction by inverting the tube a few times, followed by brief centrifugation prior to aliquotting to the reaction plate.</p> <p>Ensure that the optical lids or sealing film is completely sealed before loading qPCR instrument. This is particularly important on the edges of qPCR plates.</p>

7. Note to Purchaser: Limited License

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