



KAPA™ PROBE FAST qPCR Kit

Master Mix (2X) Bio-Rad iCycler™

1. Product Description

KAPA PROBE FAST Bio-Rad iCycler™ 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 Bio-Rad iCycler™ qPCR Master Mix (2X) is ready-to-use cocktail containing all components except primers, probe(s) and template for fast cycling probe-based real-time PCR on Bio-Rad iCycler™ iQ®, iQ™5 and MyiQ™ real-time instruments that support normalization with fluorescein reference dye at a final concentration of 10 nM. The 2X Master Mix contains KAPATaq HotStart DNA polymerase, KAPA PROBE FAST qPCR Buffer, dNTPs, MgCl₂, fluorescein reference dye and stabilizers.

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 Bio-Rad iCycler™ 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 Bio-Rad iCycler™ qPCR Kits are shipped on ice packs. Upon arrival, store all kit components protected from light at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, the 2X Master Mix will retain full activity for 18 months from the date of receipt.

3.2 Handling

Fluorescein reference dye is 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 Bio-Rad iCycler™ 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 Bio-Rad iCycler™ 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.

KK4709

 100 x 20 µl
reactions

**KAPA PROBE FAST qPCR Master Mix (2X)
Bio-Rad iCycler™ 1 x 1 ml**

 Contains:
- qPCR Master Mix (2X) with fluorescein reference dye

KK4710

 500 x 20 µl
reactions

**KAPA PROBE FAST qPCR Master Mix (2X)
Bio-Rad iCycler™ 1 x 5 ml**

 Contains:
- qPCR Master Mix (2X) with fluorescein reference dye

KK4711

 1000 x 20 µl
reactions

**KAPA PROBE FAST qPCR Master Mix (2X)
Bio-Rad iCycler™ 2 x 5 ml**

 Contains:
- qPCR Master Mix (2X) with fluorescein reference dye

 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	Fluorescein reference dye
Bio-Rad iCycler™ iQ®, iQ™5 and MyiQ™	10 nM final



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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 the Bio-Rad iCycler™ iQ®, iQ™5, and MyiQ™ Real-Time PCR Systems. This kit is not compatible with instruments that do not use fluorescein reference dye.

4.1 Step 1: qPCR Reaction Setup

- Before preparing qPCR reactions, thoroughly mix the KAPA PROBE FAST Bio-Rad iCycler™ qPCR Master Mix (2X), 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) - Bio-Rad iCycler™	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	60 °C	≥20 sec ²	

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

²Select minimum programmable time (not less than 20 sec) according to instrument guide.

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 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 Bio-Rad iCycler™ qPCR Master Mix (2X) contains fluorescein reference dye at an optimal concentration for use on the Bio-Rad iCycler™ iQ®, iQ™5 and MyiQ™ real-time instruments.

5.2 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 ABI Bio-Rad iCycler™ 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>Fluorescein was not selected as the passive reference dye at setup</p>	<p>Select fluorescein as the passive reference dye when setting up the plate.</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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