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Product Information

KiCqStart™ Probe qPCR ReadyMix™

Catalog Number **KCQS04, KCQS05, KCQS06**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

KiCqStart Probe qPCR ReadyMix is an advanced qPCR reagent system for both fast and conventional PCR cycling protocols or instruments. It is a versatile and robust solution that provides the ultimate sensitivity and high PCR efficiency using a variety of fluorogenic probe chemistries, including TaqMan® hydrolysis probes. KiCqStart Probe qPCR ReadyMix is provided as a 2X concentrated ready-to-use reaction cocktail that contains all required reaction components, except primers, probe(s), and DNA template. The light blue color of an inert tracer dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. It does not interfere with qPCR performance or affect the stability of the product.

A key component of KiCqStart Probe qPCR ReadyMix is an ultra-pure, processive thermostable DNA polymerase that is free of detectable E. coli DNA. KiCqStart Probe qPCR ReadyMix is ideal for demanding qPCR applications such as bacterial pathogen detection where residual host DNA in typical recombinant enzyme preparations can limit assay sensitivity and obscure detection of low copy samples. The enzyme in KiCqStart Probe qPCR ReadyMix is combined with high avidity monoclonal antibodies to provide a stringent automatic hot-start that allows reaction assembly, and temporary storage, at room temperature prior to PCR amplification.

Reagents

2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, TTP), hot-start DNA polymerase, ROX reference dye (if applicable), inert blue qPCR dye, and stabilizers

Product Name*	Catalog Number	SKU**	Reagent Volume
KiCqStart Probe qPCR ReadyMix	KCQS04	250RXN	2.5mL
		1250RXN	12.5mL
		5000RXN	50mL
KiCqStart Probe qPCR ReadyMix, low ROX	KCQS05	250RXN	2.5mL
		1250RXN	12.5mL
		5000RXN	50mL
KiCqStart Probe qPCR ReadyMix, ROX	KCQS06	250RXN	2.5mL
		1250RXN	12.5mL
		5000RXN	50mL

*Refer to Instrument Compatibility Section to select appropriate reagent
**Reaction number based upon a 20 µL final reaction volume

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

KiCqStart Probe qPCR ReadyMix is stable for 2 years when stored in a constant temperature freezer at –20 ºC. For convenience, it may be stored unfrozen at 2-8 ºC for up to 6 months. After thawing, mix thoroughly before using. Repeated freezing and thawing does not affect PCR performance.

Instrument Compatibility
Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. The ROX dye used in these reagents (where noted) has an excitation wavelength of ~490 nm and an emission channel of 605-610 nm. It is critical to match the appropriate qPCR reagent to your specific instrument. Please consult the following table, or visit our web site at www.sigmaaldrich.com/pcrselection to find the optimal kit for your instrument platform.

Catalog Number	Product Name	Compatible Instruments
KCQS04	KiCqStart Probe qPCR ReadyMix	Bio-Rad CFX384™, Bio-Rad CFX96™, Bio-Rad MiniOpticon™, Bio-Rad/MJ Chromo4™, Bio-Rad/MJ Opticon 2, Bio-Rad/MJ Opticon®, Cepheid SmartCycler®, Eppendorf Mastercycler® ep realplex, Eppendorf Mastercycler ep realplex2 s, Illumina Eco qPCR, Qiagen/Corbett Rotor-Gene® 3000, Qiagen/Corbett Rotor-Gene 6000, Qiagen/Corbett Rotor-Gene Q, Roche LightCycler™ 480
KCQS05	KiCqStart Probe qPCR ReadyMix, Low ROX	Applied Biosystems 7500, Applied Biosystems 7500 Fast, Applied Biosystems ViiA 7, Stratagene Mx3000P®, Stratagene Mx3005P™, Stratagene Mx4000™
KCQS06	KiCqStart Probe qPCR ReadyMix, ROX	Applied Biosystems 5700, Applied Biosystems 7000, Applied Biosystems 7300, Applied Biosystems 7700, Applied Biosystems 7900, Applied Biosystems 7900 HT Fast, Applied Biosystems 7900HT, Applied Biosystems StepOnePlus™, Applied Biosystems StepOne™

Supplies
- KiCqStart Probe qPCR ReadyMix (KCQS04, KCQS05 or KCQS06 – select appropriate reagent based upon qPCR instrument used)
- Forward and reverse primers diluted to working concentration (10 µM working stocks are sufficient for most assays)
    - Custom oligos can be designed using OligoArchitect (visit sigma.com/oligos)
- Probes diluted to working concentration (10 µM working stocks are sufficient for most assays)
    - Custom probes can be designed using OligoArchitect (visit sigma.com/probes)
- Sterile filter pipette tips
- Sterile 1.5 mL screw-top microcentrifuge tubes, such as Cat. No. CLS430909
- PCR tubes, select tubes to match desired format:
    - Individual thin-walled 200 µL PCR tubes, Cat. No.Z374873 or P3114
    - Plates
        - 96 well plates, Cat. No. Z374903
        - 384 well plates, Cat. No. Z374911
    - Plate seals
        - ThermalSeal RTS™ Sealing Films, Cat. No. Z734438
        - ThermalSeal RT2RR™ film, Cat. No. Z722553
- PCR grade water, Cat. No. W1754

Usage Guidelines
- Primer and Probe Design
    - The design of highly specific primers and probes is a critical parameter for successful qPCR. Use of Sigma's OligoArchitect™ program is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combinations. (visit sigma.com/probedesignonline)
    - For best results, amplicon size should be between 65 and 200 bp. Optimal results may require titration of primer concentration between 100 and 900 nM. A final concentration of 300 nM each primer and 100 to 250 nM probe is effective for most applications.
    - Increasing the concentration of the primer that initiates synthesis of the target strand that is complementary to the probe can improve fluorescent signal for some primer/probe systems.

- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA) and dispense equal aliquots into each reaction tube. Add the DNA template to each reaction as the final step. Addition of samples as 2 to 5-µL volumes will improve assay precision.
- Suggested input quantities of template are: cDNA corresponding to 1 pg to 100 ng of total RNA; 10 pg to 1 µg genomic DNA
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Reagent	**Volume for 20 µL reaction**	**Final Concentration**
KiCqStart Probe qPCR Readymix (2X)	10.0 µL	1X
Forward Primer	variable	100 – 900 nM (start with 300 nM)
Reverse Primer	variable	100 – 900 nM (start with 300 nM)
Probe	variable	100 – 250nM (start with 150nM)
Nuclease-free water	variable	
Template	2 to 5 µL	variable
Final Volume (µL)	20 µL	

For smaller or larger reaction volumes scale all components proportionally.

After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

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qPCR Cycling Protocol

Incubate complete reaction mix in a real-time PCR detection system as follows:

	Fast 2-Step Cycling	Fast 3-Step Cycling	Standard Cycling
Initial denaturation	95˚C, 30s*	95˚C, 30s*	95ºC, 2-3 min *
PCR cycling (30 - 45 cycles)	95ºC, 3-5s	95ºC, 3-5s	95ºC, 10-15s
		55 to 65ºC, 15s	
	60ºC, 20-30s †	68 to 72ºC, 10s †	60ºC, 30-60s †

The appropriate step for fluorescent data collection varies for different probe assay formats. Data collection for 5'-nuclease probe assays (TaqMan probe) should be carried out at the end of the extension step. Use the annealing step for data collection with hybridization probe assays (HybProbe® FRET hybridization probes, Molecular Beacons, Solaris® qPCR Assays, Scorpions® primers, etc.).
End-point analysis should be carried out at a suitable temperature for your detection probe chemistry.

* Full activation of the DNA polymerase occurs within 10 seconds at 95 ºC; however, optimal initial denaturation time is template dependent and will affect qPCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled plasmid DNA targets may require 5 to 10 min at 95 ºC to fully denature and fragment the template. Short double-stranded DNA template (PCR product) or single-stranded DNA template, such as cDNA, may require as little as 1s at 95 ºC. Use 30s at 95 ºC as a general starting point.

† Extension time is dependent upon amplicon length and the minimal data collection time requirement for your qPCR instrument. Use 30s at 60 ºC as a general starting point. Some assay designs and/or detection chemistries may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set and real-time instrument.

Quality Control

Kit components are free of contaminating DNase and RNase. KiCqStart Probe qPCR ReadyMix is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range (R2 > 0.990) with a 2-fold discrimination of starting template and a PCR efficiency > 95%. Testing of bulk enzyme for residual E. coli genomic DNA is validated to be less than 1 copy / unit.

Limited Label Licenses

The use of certain types of fluorogenic probes with 5' nuclease assays may be covered by one or more of the following US patents and corresponding patent claims outside the US: U.S. Patent No. 5,538,848, owned by Life Technologies, Corporation. Purchase of this product does not convey rights to practice the methods claimed in U.S. Patent No. 5,538,848, and a license to practice those methods must be obtained from Life Technologies, 850 Lincoln Center Drive, Forest City, California 94404, or by purchase of fluorogenic probes from an authorized source. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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