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Product Information

KiCqStart™ One-Step Probe RT-qPCR ReadyMix™

Catalog Number **KCQS07, KCQS08, KCQS09**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

KiCqStart One-Step Probe RT-qPCR ReadyMix is a ready-to-use, highly sensitive master mix for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using hybridization probe detection chemistries such as TaqMan® 5'-hydrolysis probes on real-time PCR systems. Please refer to instrument compatibility section to select the appropriate reagent for your machine. First-strand cDNA synthesis and PCR amplification are carried out in the same tube without opening between procedures. It is ideal for highly sensitive quantification of RNA viruses or low abundance RNA targets as well as high throughput gene-expression studies. The system has been optimized to deliver maximum RT-PCR efficiency, sensitivity, and specificity in reduced reaction volumes and fast cycle times.

KiCqStart One-Step Probe RT-qPCR ReadyMix contains all required components for RT-qPCR except RNA template and probe. It is compatible with all dual-labeled probe chemistries. The reverse transcriptase is an engineered M-MLV with reduced RNase H activity and improved activity and stability at higher temperatures. The use of higher temperatures (50-55 °C) for the first-strand step of one-step RT-qPCR provides higher specificity for primer annealing and disruption of RNA secondary structure that can interfere with cDNA synthesis.

KiCqStart One-Step Probe RT-qPCR ReadyMix is highly resistant to PCR inhibitors. A key component of the ReadyMix is an ultra-pure, highly processive, thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This provides an extremely stringent automatic hot-start that minimizes the potential for primer-dimer and other non-specific PCR artifacts. The light blue color of the inert dye simplifies reaction assembly in white, or clear, plates and helps to minimize pipetting or mixing errors. The dye does not interfere with qPCR performance or affect the stability of the product.

Reagents

2X reaction buffer containing dATP, dCTP, dGTP, TTP, magnesium chloride, reverse transcriptase, RNase inhibitor protein, hot-start DNA polymerase, ROX reference dye (if applicable), inert blue qPCR dye, and stabilizers

Product Name*	Catalog Number	SKU**	Reagent Volume
KiCqStart One-Step Probe RT-qPCR ReadyMix	KCQS07	500RXN	5mL
KiCqStart One-Step Probe RT-qPCR ReadyMix, Low ROX	KCQS08	500RXN	5mL
KiCqStart One-Step Probe RT-qPCR ReadyMix, ROX	KCQS09	500RXN	5mL

*Refer to Instrument Compatibility Section to select appropriate reagent
**Reaction number based upon a 20µL 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 One-Step Probe RT-qPCR ReadyMix is stable for one year when stored in a constant temperature freezer at -20 ºC, protected from light. Repeated freezing and thawing does not affect RT-qPCR 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. 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
KCQS07	KiCqStart One-Step Probe RT-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
KCQS08	KiCqStart One-Step Probe RT-qPCR ReadyMix, Low ROX	Applied Biosystems 7500, Applied Biosystems 7500 Fast, Applied Biosystems ViiA 7, Stratagene Mx3000P®, Stratagene Mx3005P™, Stratagene Mx4000™
KCQS09	KiCqStart One-Step Probe RT-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™ One-Step Probe RT-qPCR ReadyMix™ (KCQS07, KCQS08 or KCQS09 – select appropriate reagent based upon qPCR instrument used)
- Forward and reverse primers diluted to working concentration (10µM working stocks are sufficient for most assays)
    - Custom oligos can be designed using OligoArchitect (visit sigma.com/oligos)
- Probes diluted to working concentration (10µM 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 one-step RT-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)
    - Regions of strong RNA secondary structure should be avoided as this can interfere with primer hybridization and/or impede procession of the reverse transcriptase.
    - For best results, amplicon size should be between 70 and 150 bp. Optimal results may require titration of primer concentration between 300 and 900 nM. A final concentration of 450 nM each primer and 150 nM probe is effective for most applications.
    - The efficacy and efficiency of any primer/probe set should be validated under fast cycling and/or rapid ramp rate protocols before use in qPCR studies.

- If frozen, thaw KiCqStart One-Step Probe RT-qPCR ReadyMix on ice. Thoroughly mix by gently vortexing, and then centrifuge to collect contents to the bottom of the tube. Keep on ice prior to use.

- To maximize specificity, reactions should be assembled on ice. The hot-start DNA polymerase is inactive prior to high temperature activation; however, the reverse transcriptase is active at lower temperatures.

- First-strand synthesis can be carried out between 42 °C and 55 °C. Optimal results are generally obtained with a 5 to 10-minute incubation at 48-50 °C. Longer incubation times for first-strand synthesis (up to 20 min) may be used.

- We recommend a minimum of 30s incubation at 95 °C to inactivate the RT and activate the hot-start polymerase prior to PCR cycling.

- The kit is compatible with either fast or standard qPCR cycling protocols. Annealing and or extension temperatures may need to be optimized for a given primer/probe design or fluorogenic probe chemistry. Use the suggested protocol as a starting point. Multiplexed RT-qPCR may benefit from a slightly longer extension time (45-60s).

- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except RNA template and dispense equal aliquots into each reaction tube. Add RNA to each reaction as the final step. Addition of sample as 2 to 5-µL volumes will improve assay precision.

- Suggested input quantities of template are: 1 pg to 100 ng total RNA; 10 fg to 10 ng poly A(+) RNA; 10 to $1x10^8$ copies viral RNA.

- 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 One-Step Probe RT-qPCR (2X)	10.0 µL	1X
Forward Primer	variable	300 – 900 nM (start with 450 nM)
Reverse Primer	variable	300 – 900 nM (start with 450 nM)
Probe	variable	50 – 200nM (start with 150nM)
Nuclease-free water	variable	
RNA Template	2 to 5 µL	variable
Final Volume (µL)	20 µL	

Final reaction volume may vary from 10 to 50 µL, 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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RT-qPCR Cycling Protocol

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

cDNA Synthesis	50 °C, 10 min
Initial denaturation	95 °C, 1 min
PCR cycling (30 - 45 cycles)	95 °C, 3-10s 60°C, 30-60s (data collection step)

Quality Control

Kit components are free of contaminating DNase and RNase. KiCqStart™ One-Step Probe RT-qPCR ReadyMix™ is functionally tested in RT-qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range (r^2> 0.995) and a PCR efficiency > 90%.

Limited Label Licenses

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