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Product Information

KiCqStart™ SYBR® Green Primers

Catalog Number **KSPQ12012**
Storage Temperature -20ºC

Technical Bulletin

Product Description
KiCqStart Primers include one forward and one reverse primer, dry, in individual tubes. The primers consist of DNA bases, have no modifications, are reverse-phased purified, and each comes with a minimum quantity of 3 OD. For sequence information, see the Technical Data Sheets that come with every primer pair.

Product Use
KiCqStart Primers are intended to be used for quantifying gene expression with two-step and one-step SYBR Green I RT-qPCR (reverse transcription quantitative real-time PCR). Though co-amplification of other transcripts is possible, they have been designed to detect the most prevalent mRNA splice variant in eukaryotes for each gene with amplicon sizes ranging between 75 and 200 base pairs.

For optimum results, use KiCqStart Primers with Sigma's ReadyScript™ cDNA Synthesis Mix, Catalog Number RDRT, and KiCqStart SYBR Green qPCR ReadyMix™, Catalog Number KCQS00, for two-step reactions.

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 Primers should be stored dry or as wet single-use aliquots at −20 ºC.
Avoid repeated freeze-thaw cycles.

Preparation
- KiCqStart Primers should be briefly centrifuged and then resuspended in a weak buffer, such as TE (10 mM Tris, pH 7.5, 1 mM EDTA, diluted from Catalog Number T9285). If TE is not suitable, PCR grade water, Catalog Number W1754, is the next best choice.

Number of Reactions
KiCqStart Primers typically function best with a final concentration of each primer at 450 nM in a final reaction volume of 20 µL. With these specifications, the 3 OD minimum quantity of each primer (assuming 21mer with an average MW = 6363) is enough for 1,500 reactions.

qPCR Standard Protocol

The following is a protocol that can be used as a basic template for modification or as a quick check for a set of primer pairs. Primers are used at a final concentration of 450 nM and are run in SYBR Green I master mix.

Equipment
- Real-time PCR instrument

Reagents
- cDNA diluted 1:10 or gDNA
- KiCqStart SYBR Green ReadyMix, Catalog Numbers KCQS00/ KCQS01/ KCQS02/ KCQS03; instrument specific
- PCR grade water, Catalog Number W1754
- Forward and reverse primers for test gene (stock at 100 µM)

Supplies
- Laminar flow hood for PCR set up (optional)
- 1.5 mL tubes, sterile
- Tube racks for 1.5 mL tubes
- PCR tubes
- Caps for PCR tubes
- Pipettes
- Aerosol-barrier pipette tips

Safety
- Lab coats
- Gloves
- Eye protection (safety glasses)

Notes

- cDNA is generated using random / oligo-dT priming and is diluted 1:10 to 1:100 for use in qPCR
- Forward and reverse primers for the test gene are assumed to be 10 µM stocks
- All reactions are run in duplicate as technical replicates

Method

1. Prepare a master mix for all reactions in duplicate and 2x NTC (no template control) according to the table below (calculate volumes for each reaction and add 10%). Mix well, avoiding bubbles.

Reagent	Volume per single 20 µL reaction
KiCqStart SYBR Green qPCR ReadyMix 2X	10 µL
Forward primer (10 µM stock)	0.9 µL
Reverse primer (10 µM stock)	0.9 µL
PCR grade water	4.2 µL

2. Remove 32 µL of master mix from #1 and place into a separate tube for the NTC reactions.
3. Add 8 µL of water to the NTC master mix from step #2. Set NTC master mix on ice.
4. Aliquot 4 µL of diluted cDNA into the bottom of the PCR tube (check that all wells contain the correct volume)
5. Carefully aliquot 16 µL of template master mix remaining from step #2 into the PCR plate (taking care not to come into contact with the sample; change tips if required).
6. Aliquot 20 µL of NTC master mix from step #3 into the PCR plate.
7. Cap tubes, label, and spin plates.

Note: Make sure that the labeling does not interfere with the instrument excitation and detection.

8. Run samples according to the 3-step protocol in the table below (conditions are specific for FAST or Standard cycling conditions):

FAST Cycling Conditions		
	Temp (°C)	Time
Step 1	95	30
Step 2 (40 cycles)	95	5
	58	15
	72	10
Standard Cycling Conditions		
	Temp (ºC)°	Time
Step 1	95	30
Step 2 (40 cycles)	95	15
	58	30
	72	15

Use a standard dissociation curve protocol (data collection).

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ReadyMix and ReadyScript are trademarks of Sigma-Aldrich Co. LLC
SYBR is a registered trademark of Molecular Probes, Inc.

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