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Product Information

KiCqStart® SYBR® Green Primers Gene Arrays

Catalog Number **KSGA2013**
Storage Temperature –20 °C

Technical Bulletin

Product Description
KiCqStart Primers Gene Arrays include mixed forward and reverse primers for each gene in the array, dry, in individual 96 or 384 plate wells. Depending on the number of primer pairs in the array and the plate type, there can be anywhere from one to more than 10 plates per order. The primer pairs consist of DNA bases, have no modifications, are reverse-phased purified, and come in a final quantity of 6 nmol (3 nmol for each primer). For sequence information, see the spreadsheet that comes with every order.

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 PCR plates at −20 ºC.
Avoid repeated freeze-thaw cycles.

Preparation
KiCqStart Primers Gene Arrays 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. The primer pairs must be transferred to PCR plates for use.

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 6 nmol quantity of each primer pair is enough to prepare over 300 individual PCR plates (reactions).

qPCR Standard Protocol
The following is a protocol that can be used as a basic template and adapted as required, according to optimization 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 KiCqStart 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 genes are resuspended to 20 µM stocks
- All reactions are run in duplicate as technical replicates

Method
1. For 96-well plates, add 300 µL dH_2O to each well containing the primer pairs. For 384-well plates, perform a serial dilution to achieve a final concentration of 20 µM in each well of the final stock plate.
2. Transfer 0.9 µL to the destination PCR plate.
3. Prepare a master mix for all reactions (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
PCR grade water	5.1 µL
Template, e.g., cDNA diluted 1:10/1:100 or PCR grade water (if running a NTC control plate)	4 µL

4. Carefully aliquot 19.1 µL of template master mix from step 3. into the PCR plate (taking care not to come into contact with the primers; change tips if required).
5. Cover the plate wells according to the instrument requirements.
6. Centrifuge to collect sample to the bottom of the tubes.

Notes:
- It is advisable to run a control plate including water in the mastermix, in place of cDNA template.
- Make sure that the labeling does not interfere with the instrument excitation and detection.

7. Run samples according to the standard SYBR Green protocol for the instrument or 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 (sec)
Step 1	95	30
Step 2 (40 cycles)	95	5
	58	15
	72	10
Standard Cycling Conditions		
	Temp (°C)	Time (sec)
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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