

Product Information

KiCqStart® Probe Assays

Catalog Number **KSPRA2015**

Storage Temperature –20 °C

Technical Bulletin

Product Description

KiCqStart Probe Assays work via the 5' nuclease assay and include one forward primer, one reverse primer, and one Dual-Labeled (hydrolysis) Probe. All are provided dry in individual tubes.

The primers consist of DNA bases:

- no modifications
- reverse-phased purified
- minimum quantity of 3 OD

The probe consists of DNA bases:

- no internal modifications
- labeled with either 5' 6-FAM™ or HEX™
- also labeled with 3' Onyx Quencher™ A (OQ™A—see sigma.com/darkquencher)
- HPLC purified
- quantity of 1 OD

For sequence information, see the Technical Datasheet that comes with every assay.

Product Use

KiCqStart Probe Assays are intended to be used for quantifying gene expression with two-step and one-step RT-qPCR (reverse transcription quantitative real-time PCR). They have been designed to detect the most prevalent mRNA splice variant in eukaryotes for each gene with amplicon sizes ranging between 75 and 150 bases.

For optimum results, use KiCqStart Probe Assays with Sigma's ReadyScript™ cDNA Synthesis Mix, Catalog Number RDRT, and KiCqStart Probe qPCR ReadyMix™, Catalog Number KCQS04, 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 Safety Data Sheet for information regarding hazards and safe handling practices.

Storage / Stability

KiCqStart Probe Assays reagents should be stored dry or as wet single-use aliquots at –20 °C. Avoid repeated freeze-thaw cycles.

Preparation

KiCqStart Probe Assays reagents 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, sterile, nuclease-free water, Catalog Number W4502, is the next best choice.

10 µM stock solutions are recommended.

Number of Reactions

KiCqStart Probe Assays typically function best with a final concentration of the primers at 300 nM and the probe at 200 nM in a final reaction volume of 20 µL. With these specifications, there is enough material for 850 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 an assay. Primers and probe are used at a final concentration of 300 and 200 nM, respectively, and are run in probe master mix.

Equipment

- Real-time PCR instrument

Reagents

- cDNA diluted 1:10 or gDNA
- KiCqStart Probe qPCR ReadyMix, Catalog Numbers KCQS04/ KCQS05/ KCQS06, instrument specific
- PCR grade water, Catalog Number W1754
- Forward primer, reverse primer, and probe 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 primer, reverse primer, and probe for the test gene are assumed to be 10 μ M 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 μ L reaction
KiCqStart Probe qPCR ReadyMix 2X	10 μ L
Forward primer (10 μ M stock)	0.6 μ L
Reverse primer (10 μ M stock)	0.6 μ L
Probe (10 μ M stock)	0.4 μ L
PCR grade water	4.4 μ L

2. Remove 32 μ L of master mix from #1 and place into a separate tube for the NTC reactions.
3. Add 8 μ L of water to the NTC master mix from step #2. Set NTC master mix on ice.
4. Aliquot 4 μ L of diluted cDNA into the bottom of the PCR tube (check that all wells contain the correct volume)
5. Carefully aliquot 16 μ L 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 μ L 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 2-step protocol in the table below:

Cycling Conditions		
	Temp ($^{\circ}$ C)	Time (s)
Step 1	95	20
Step 2 (40 cycles)	95	5
	58	30

Use a standard dissociation curve protocol (data collection).

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