PhileKorea

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Product Insert

QuantiSpeed Probe No-Rox Kit

Cat. No.
QS755-02 200 x 20ul reaction: 2 x 1ml
QS755-05 500 x 20ul reaction: 5 x 1ml
QS755-10 1000 x 20ul reaction: 10 x 1ml

Shipping: On Dry/Blue Ice
Store at -20℃
For research use only.

Storage and Stability

The QuantiSpeed Probe No-ROX Kit is shipped on Dry/Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. When stored under optimum conditions, the reagents are stable for a minimum of 12 months from date of purchase.

Quality Control

The QuantiSpeed Probe No-ROX Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety Precautions

Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Description

The QuantiSpeed Probe No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry and enhancers, together with an antibody-mediated hot-start DNA polymerase system, ensures that the QuantiSpeed Probe No-ROX Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.
QuantiSpeed Probe No-ROX is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs, stabilisers and enhancers.

Kit Components

Reagent	200x20ul	500x20ul	1000x20ul
QuantiSpeed Probe No-ROX Mix (2x)	2x1ml	5x1ml	10x1ml

Instrument compatibility

The QuantiSpeed Probe No-ROX Kit is compatible with real-time instruments that do not need a passive reference signal for normalization of the data. The QuantiSpeed Probe No-ROX Kit has been optimized for use on the real-time instruments listed in the following compatibility table.

Manufacturer	Model
Illumina®	Eco™
Bio-Rad	iCycler®, MyiQ®, iQ™5, Opticon™, Opticon2™, MiniOpticon,Chromo4™, CFX96, CFX384
Roche	LightCycler® 480, LightCycler®Nano
Qiagen	Rotor-Gene™ 3000 & 6000 & Q
Takara	Thermal Cycler Dice® (TP800)
Eppendorf	Mastercycler®, ep realplex, Mastercycler®realplex25
Cepheid	SmartCycler™
Techne	Quantica®
Analytica Jena	qTower
Applied Biosystems	7500, 7500FAST, Viia7™
Stratagene(Agilent)	MX4000P®, MX3000P®, MX3005P®

General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction setup, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers and Probe: These guidelines refer to the design and set up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe type. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the primers, as well as by the amplicon length.
We strongly recommend taking the following points into consideration when designing and running your real-time PCR:

- use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMPTM (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C; the Tm of the Probe should be approximately 10°C higher than that of the primers.
- optimal amplicon length should be 80-200bp, and should not exceed 300bp
- final primer concentration of 400nM is suitable for most Probe based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1μM. The forward and reverse primers concentration should be equimolar
- a final probe concentration of 100nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration

Note: Multiplex real-time PCR probe concentrations in excess of 100nM, can result in cross channel fluorescence

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following points should be considered when using genomic DNA and cDNA templates:

- **Genomic DNA:** use up to 1ug of complex (e.g. eukaryotic) genomic DNA in a single PCR. We recommend using the PKT Xprep DNA Mini Kit for high yield and purity from both prokaryotic and eukaryotic sources
- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene. We suggest using 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using the PKT cDNA Synthesis Kit for reverse transcription of the purified RNA. For high yield and purity of RNA, use the PKT Xprep RNA Mini Kit.

$MgCl_2$: The QuantiSpeed Probe mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control (NTC), replacing the template with PCR grade water. When performing a two-step RTPCR, set up a no-RT control as well as an NTC for the PCR.

Procedure

Reaction mix composition:
Prepare a PCR mastermix. The volumes given below are based on a standard 20ul final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x QuantiSpeed Probe No-ROX Mix	10ul	1x
10uM Forward Primer	0.8ul	400nM
10uM Reverse Primer	0.8ul	400nM
10uM Probe	0.4ul	200nM
Template	<100ng cDNA, <1ug genomic	variable
H_2O	As required	
	20ul Final volume	

Sensitivity testing and Ct values:
When comparing QuantiSpeed with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed.

Suggested thermal cycling conditions:
The following real-time PCR conditions are suitable for the QuantiSpeed Probe No-ROX Kit with the amplicons of up to 200bp. These cycling paramenters have been optimized on a number of platform, however they can be varied to suit different machine-specific protocols.

Cycles	Temperature	Time	Notes
1	*95℃	*2min	Polymerase activation
40	95℃	5s	Denaturation
	60-65℃	**20-30s	Annealing/extension (acquire at end of step)

*** 2min for cDNA, 3min for genomic DNA**
**** Not recommended to anneal/extend beyond 30 seconds**

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant date.

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