PhileKorea
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Product Insert

QuantiSpeed SYBR No-Rox Kit

Cat. No.
QS105-02 200 x 20ul reaction: 2 x 1ml
QS105-05 500 x 20ul reaction: 5 x 1ml
QS105-10 1000 x 20ul reaction: 10 x 1ml

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

Storage and Stability

The QuantiSpeed SYBR 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 SYBR 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 swalNoed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Description

The QuantiSpeed SYBR No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time instruments. 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 SYBR No-ROX Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.
For ease-of-use and added convenience, QuantiSpeed SYBR No-ROX is provided as a 2x mastermix containing all the components necessary for real-time PCR, including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. The kit consists of a ready-to-use premix, only primers and template need to be added.

Kit Components

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

Kit compatibility

The QuantiSpeed SYBR No-ROX Kit has been optimized for use in SYBR Green-based real-time PCR on the real-time instruments listed in the folNoing compatibility table, each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Qiagen (Corbett) Rotor-Gene™ 3000&6000&Q, the Bio-Rad CFX96 or the Roche LightCycler® 480.

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: 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 folNoing 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.
- optimal amplicon length should be 80-200bp, and should not exceed 400bp
- final primer concentration of 400nM is suitable for most SYBRGreen based reactions, however to determine the optimal concentration we recommend titrating in the range 0.1-1μM
- use an equimolar primer concentration
- when amplifying from cDNA,use of intron spanning primers to is preferable, to avoid amplification from genomic DNA

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 folNoing 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 $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of real-time PCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase.

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.

Optional ROX: The QuantiSpeed SYBR No-ROX Kit is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software.

Procedure

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

Reagent	Volume	Final concentration
2x QuantiSpeed SYBR No-ROX Mix	10ul	1x
10uM Forward Primer	0.8ul	400nM
10uM Reverse Primer	0.8ul	400nM
H_2O	Up to 20ul	
Template	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 No template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of speed. In some instances increasing final $MgCl_2$ concentration to 6mM will reduce Cts for difficult amplicons.

Suggested thermal cycling conditions:
The folNoing real-time PCR conditions are suitable for the QuantiSpeed SYBR No-ROX Kit with the amplicons of up to 200bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures beNo 60°C or combined annealing/extension times longer than 30 seconds.

QuantiSpeed SYBR No-ROX Kit is compatible

- **2-step cycling**

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**

Optional analysis: After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.

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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