Perfectstart SYBR Green qPCR master mix

Shipping and storage

Kit components are shipped at 2-8℃, long term storage at -20℃. Minimize exposure of both the mix and ROX reference dye to direct light. Exposure to direct light for an extended period of time may result in loss of fluorescent signal intensity.

Description

The Omega Bio-Tek's perfectstart SYBR Green qPCR master mix is optimized to amplification and detection of DNA in real-time quantitative PCR (qPCR). It is a ready-to use mix containing all components, except primers and template. It combines a chemically modified "hot-start" version of Taq DNA polymerase and a novel fluorescent dye to deliver excellent sensitivity in the quantification of target sequences, with a linear dose response over a wide range of target concentrations. Volumes are provided for 100 or 400 amplification reactions of 25 µl each.

The Omega Bio-Tek's perfectstart SYBR Green qPCR master mix is supplied at a 2×concentration and contains "hot-start" Taq DNA polymerase, SYBR Green I fluorescent dye, Mg^{2+}, dNTPs and stablilizers. The master mix can quantify fewer than 10 copies of target gene, has a broad dynamic range, and is compatible with melting curve analysis.

- The SYBR Green I dye fluorescent double-stranded DNA binding dye in the master mix provides both higher sensitivity and lower PCR inhibition.
- The Taq DNA polymerase provided in the master mix has been chemically modified to block polymerase activity at ambient temperatures, allowing room-temperature setup and long-term storage at 4℃. Activity is restored after a 10 min incubation in PCR cycling, providing an automatic hot start for increased sensitivity, specificity, and yield.
- ROX reference dye is included as a separate component to normalize the fluorescent signal between reactions for instruments that are compatible with this option. ROX can adjust for non-PCR-related fluctuations in fluctuations in fluorescence between reactions, and provides a stable baseline in multiplex reactions.The volume of 50x Rox to add to the qPCR reaction can be adjusted as instrument required.

Kit components

Cat.No.	TQ2300-01	TQ2300-02
Preps	100 preps	400 preps
2 × perfectstart SYBR Green qPCR master mix	1.25 ml	1.25 ml×4
50×ROX Reference Dye	50 µl	200 µl

Important Parameters

Instrument compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the ABI 7300 and 7500 real-time PCR systems; the Bio-Rad iCycler; the Stratagene Mx3000P; the MJ research Chromo 4 real-time detector. Optimal cycling conditions will vary with different instruments.

Template

cDNA

- For two-step qRT-PCR, use undiluted or diluted cDNA generated from up to 1 µg of total RNA, for cDNA synthesis, we recommend the Omega Bio-Tek's M-MLV Frist Strand cDNA Synthesis Kit.
- A maximum of 10% of the qPCR reaction volume may be undiluted cDNA.
- Note that detecting high-abundance genes in undiluted cDNA may result in very low CTs in qPCR, leading to reduced quantification accuracy. Prepare a dilution series of the cDNA template for the most accurate results.

Plasmid and Genomic DNA

Use up to 100 ng of genomic DNA or 10-10^7 copies of plasmid DNA in a 10 µl volume. Note that 1 µg of plasmid DNA contains 9.1 × 10^{11} copies divided by the plasmid size in kilobases.

Primers

Primer design is one of the most important parameters when using perfectstart SYBR Green qPCR master mix. When designing peimers, the amplicon length should be approximately 80-250 bp. A final concentration of 400 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 100 and 600 nM.

DNA Polymerase Activation Time

The hot-start DNA polymerase is activated in the 10 min incubation at 95℃ before PCR cycling.

Melting Curve Analysis

Melting curve analysis should always be performed following real-time qPCR to identify the presence of primer dimers and analyze the specificity of the reaction. Program your instrument for melting curve analysis using the instructions provided with your specific instrument.

General Protocol

Follow the general protocol below for qPCR on ABI real-time instruments. Note the lower amount of ROX Reference Dye required for the ABI 7300. This generic protocol may also be used as a starting point for other real-time instruments.

1. Program your real-time instrument as shown below. Optimal temperatures and incubation times may vary.

   95℃ for 10 min hold(DNA polymerase activation)

   40-45 cycles of :

   95℃ , 15s

   55-60℃, 20s

   72℃, 20s

   Melting curve analysis : Refer to instrument documentation

2. For each reaction, add the following to a 0.2 ml microcentrifuge tube or each well of a PCR plate. A standard 25 µl reaction size is provided; component volumes can be scaled as desired.

   For multiple reactions, prepare a master mix of common components, add the appropriate volume to each tube or palte well, and then add the unique reaction components.

Component	Single rxn	Final conc.
2×perfectstart SYBR Green qPCR master mix	12.5 µl	1×
Forward primer, 10 uM	1 µl	400 nM
Reverse primer, 10 uM	1 µl	400 nM
ROX Reference Dye (optional)	0.5 µl /0.05 µl	
Template	2-5 µl	
DEPC-treated water	To 25 µl	

3. Cap or seal the reaction tube/PCR plate, and gently mix. Make sure that all components are at the bottom of the tube/plate; centrifuge briefly if needed.
4. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.