RB SYBR qPCR

Description:

The **RB SYBR qPCR Kit** is supplied as a 2X concentrated ready-to-use mixture for amplification of specific DNA fragments. It can be used in place of a single reagent for real-time quantitative PCR. The mixture contains Hot Start Taq DNA polymerase and other components required for real-time quantitative PCR with the exception of template and primers. Hot Start Taq DNA polymerase is complex mixture of a thermostable Taq DNA polymerase and specific monoclonal antibodies. It can eliminate amplification artifacts such as primer-dimer formation and mis-priming during preamplification stage and thus may provide improved specificity when compared to standard DNA polymerase. This not only saves valuable time in the laboratory, but also reduces the number of pipetting and reagent handling errors. A 1X RB SYBR qPCR mix contains 2 U of recombinant Hot Start Taq DNA polymerase, reaction buffer, 3 mM MgCl2, 250 µM dNTP, SYBR Green I, ROX passive reference dye, and enzyme stabilizer sufficient to allow efficient amplification of template in a 25 µl reaction.

Components of the kit :

1 tube of 1 ml RB SYBR qPCR Mix.

General Procedure:

1. Prepare the reaction mix by combining the indicated volumes of components of 2X**RB SYBR qPCR Mix**:
2.

Experimental Sample

Nuclease-free H2O	X µl
cDNA template	Y µl
Upstream primer (10 pM)	1 µl
Downstream primer (10 pM)	1 µl
2XRB SYBR qPCR Mix	12.5 µl
Total	25 µl

3. Place the tubes in a qPCR machine and proceed directly to amplify DNA. An initial denaturation step should be performed first at 94~95 °C for 5 minutes. This inactivates the antibody and initiates the hot-start PCR, then proceed a general PCR program.

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Note:

1. Typical amplified sizes for real-time quantitative PCR products are less than 300 bp.
2. For either the two-step or the three-step protocol, set the cycle numbers in the range of 30~45 cycles.

Examples of typical cycling parameters

Two-Step program

Step	Temp	Time	Detection
Denaturation	94~95℃	20~30s	off
Annealing/ Extension	TM-5℃	30s~1min	on

Three-Step program

Step	Temp	Time	Detection
Denaturation	94~95℃	20 s~30 s	off
Annealing	TM-5℃	30 s~45 s	off
Extension	72℃	30 s~1 min	on

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