RB Taq ReadyMix PCR Kit

Product Description:

RB Taq DNA Polymerase is the single-subunit, wild-type *Taq* DNA polymerase from the thermophilic bacterium *Thermus aquaticus*, produced from recombinant *Escherichia coli*. RB Taq has 5'g3' polymerase and 5'g3' exonuclease activities, but no 3'g5' exonuclease (proofreading) activity. The enzyme has an error rate of approximately 1 error per 2.2 x 105 nucleotides incorporated. PCR products generated with RB Taq are A-tailed and may be cloned into TA cloning vectors.

RB Taq ReadyMix (5X) is a ready-to-use cocktail containing all components for PCR, except primers and template. The 5X ReadyMix contains RB Taq DNA Polymerase (1 U per 50 µl reaction), RB Taq Buffer, dNTPs (0.2 mM of each dNTP at 1X), MgCl2 (1.5 mM at 1X) and stabilizers.

RB Taq PCR Protocol

RB Taq DNA Polymerase can be used to replace any commercial *Taq* DNA polymerase in an existing protocol. Due to differences in buffer formulations, it may be necessary to re-optimize annealing temperatures using RB Taq ReadyMix should existing annealing temperatures give poor results.

Step 1: Prepare the PCR master mix

Ensure that all reagents are properly thawed and mixed.

Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.

Calculate the required volumes of each component based on the following table:

Component	50 µl rxn[1]	Final conc.
PCR-grade water	Up to 50 µl	N/A
5X RB Taq ReadyMix (1.5 mM MgCl2 at 1X)[2]	10 µl	1X
5 U/µl RB Taq DNA Polymerase	0.3 µl	1.5 U
10 µM Forward Primer	0.5 – 2.5 µl	0.2 – 0.5 µM
10 µM Reverse Primer	0.5 – 2.5 µl	0.2 – 0.5 µM
Template DNA[3]	As required	As required

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[1] Reaction volumes of 10 – 50 µl are recommended. For volumes smaller than 50 µl, scale reagents down proportionally.

[2] A final MgCl2 concentration of 1.5 mM is sufficient for most standard applications. For assays that do not perform well with 1.5 mM MgCl2, the optimal MgCl2 concentration for each primer/ template combination should be determined empirically.

[3] ≤250 ng for genomic DNA; ≤25 ng for less complex DNA (e.g. plasmid, lambda).

NOTE: For GC-rich or other difficult templates or amplicons, include DMSO at a final concentration of 5%.

Step 2: Set up individual reactions

• Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells or a PCR plate

• Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95 ºC	3 min[1]	1
Denaturation	95 ºC	30 sec	35- 40[3]
Annealing[2]	T m – 5 °C	30 sec	
Extension	72 °C	1 min/kb	
Final extension (optional)[4]	72 °C	1 min/kb	1
Hold	4 – 10 °C	∞	1

[1] Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95 °C may be used.

[2] An annealing temperature 5 °C lower than the calculated melting temperature (Tm) of the primer set is recommended as a first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

[3] 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring higher sensitivity, while lower cycle numbers can be used if the template copy number is high.

[4] Final extension should be included if PCR products are to be cloned into TA cloning vectors.

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