RB Taq PCR Protocol

Product Applications

RB Taq DNA Polymerase is ideally suited for:

Routine PCR

Amplification of DNA for Sanger sequencing

Any standard PCR application for which a hot start formulation of a high-quality thermostable DNA polymerase is required.

Product Specifications

Shipping and Storage

RB Taq PCR kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -20 °C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label.

Handling

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 4 °C for short-term use (up to 1 month). Return to -20 °C for long-term storage.

Quality Control

Each batch of RB Taq DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). RB Taq PCR kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

RB Taq PCR Protocol

RB Taq DNA Polymerase can be used to replace any commercial *Taq* DNA polymerase in an existing protocol. To allow the most seamless integration of RB Taq into existing protocols, be sure to match reaction conditions, particularly the MgCl2, primer and enzyme concentrations, as closely as possible.

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 reactions to be performed.

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

Component	50 µl reaction1	Final conc.
PCR-grade water	Up to 50 µl	N/A
10X RB Taq Buffer2	5.0 µl	1X
25 mM MgCl2	As required3	>1.5 mM
10 mM dNTP Mix	1.0 µl	0.2 mM each
10 µM Forward Primer	2.0 µl	0.4 µM
5 U/µl RB Taq DNA Polymerase4	0.2 µl	1 U
Template DNA	As required	As required5

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

2 RB Taq Buffer A, RB Taq Buffer B and RB Taq Buffer with dye all contain a final MgCl2 concentration of 1.5 mM at 1X.

3 For assays requiring >1.5 mM MgCl2, the reaction may be supplemented with additional MgCl2 as required.

4 For GC-rich and other difficult templates, higher enzyme concentrations (up to 5 U per 50 µl reaction) may be required.

5 ≤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 of 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 min1	1
Denaturation	95 ºC	30 sec	353
Annealing2	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 content) 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 first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature for the primer set empirically.

3 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring a higher level of sensitivity.

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

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