HotStart DNA Polymerase

Store at -20°C

Cat. No.	Description	Concentration	Quantity
G011	HotStart DNA Polymerase	5 U/µl	250 U
G039	HotStart DNA Polymerase	5 U/µl	1000 U

Product Description

crb's HotStart DNA Polymerase contains a proprietary antibody that blocks polymerase activity at low temperatures. During the initial denaturation step at 94°C, the antibody dissociates from DNA polymerase and restores enzyme activity. This feature significantly reduces non-specific product formations that would otherwise compete for reagent availability, thus offering higher specificity and improved yield of PCR products. PCR products amplified up to 6 kb in length with HotStart DNA Polymerase contain a single base (A) 3' overhang.

Product Components	250 U	1000 U
HotStart DNA Polymerase (5 U/µl)	50 µl	200 µl
10X PCR buffer, with Mg^{2+}	1 ml	3 ml
25 mM $MgSO_4$	1 ml	1 ml

Storage Buffer Components

50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50 % glycerol and 1.0 % Triton®X-100.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 mins at 70°C.

Shipping and Storage

Upon arrival, HotStart DNA Polymerase should be stored at -20°C. Avoid repeated freeze-thaw cycles of all HotStart components to retain maximum performance.

Protocol

The following basic protocol serves as a general guideline and starting point for any PCR amplification. Optimal reaction conditions (incubation times, temperatures, concentration of HotStart DNA Polymerase, primers, $MgSO_4$ and template DNA) may vary and need to be optimized for each specific PCR.

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination.

A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µl
Forward primer (10 µM)	1 - 2.5 µl	200 - 500 nM
Reverse primer (10 µM)	1 - 2.5 µl	200 - 500 nM
10X PCR buffer, with Mg^{2+}	5 µl	1X
25 mM $MgSO_4$ (optional)*	0 - 3 µl	1.5 - 3 mM
dNTP Mix (10 mM)	1 µl	200 µM
HotStart DNA Polymerase (5 U/µl)	0.5 - 1 µl	2.5 - 5 U
Nuclease-free H_2O	up to 50 µl	-

* *Optimal Mg^{2+} concentration is specific to each DNA template-primer set and can only be determined experimentally.*

• *We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.*

2. Mix contents of tube and centrifuge briefly.
3. Incubate tube in a thermal cycler at 94°C for 10 mins to completely activate the HotStart DNA Polymerase and denature the template.
4. Perform 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 30 secs

   Anneal: 45 - 72°C for 30 secs

   Extend: 72°C for 1 min/1 kb template

5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat No. G108) staining. Use appropriate molecular weight standards.

All **crb** PCR, RT-PCR, and qPCR products are ISO 13485:2003 and13485:2012 certified as diagnostic grade and in compliance with all regulatory requirements for the design and manufacture of medical devices, as outlined by the International Organization for Standardization (ISO). For technical questions, please email us at support@coderegenesis.com or visit our website at www.coderegenesis.com.

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