TaqFast DNA Polymerase

Store at-20°C

Cat. No.	Description	Concentration	Quantity
G277	TaqFast DNA Polymerase	5 U/µl	250 U
G278	TaqFast DNA Polymerase	5 U/µl	1000 U

Product Description

TaqFast DNA polymerase is an engineered version of Taq DNA polymerase developed to achieve rapid PCR. It catalyzes the 5′-3′ synthesis of DNA. This enzyme possesses 5′-3′ exonuclease activity and moderate 3′-5′ proofreading exonuclease activity. The extension speed is about 6 kb/min, which is 6 times faster than the regular Taq DNA polymerase. TaqFast DNA polymerase generates blunt-end PCR products, but a template-independent "A" can be attached at the 3′ end of the PCR product which can then be cloned into a TA cloning vector.

Product Components	250U	1000U
TaqFast DNA Polymerase (5 U/µl)	50 µl	200 µl
5X PCR Buffer, with Mg^{2+}	1 ml	4 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 the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 mins at 74°C.

Shipping and Storage

Upon arrival, TaqFast DNA Polymerase should be stored at -20°C. Avoid repeated freeze-thaw cycles of all TaqFast components to retain maximum performance. All TaqFast components are stable for 1 year from the date of shipping if stored and handled properly.

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 Taq 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 (10µM)	1 - 2.5 µl	200 - 500 nM
5X PCR Buffer, with Mg^{2+}	10 µl	1X
25 mM $MgSO_4$ (optional)*	0 - 3 µl	1.5 - 3 mM
dNTP Mix (10 mM)	1 µl	200 µM
TaqFast DNA Polymerase (5 U/µl)	0.5 - 1 µl	2.5-5U
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 3 mins to completely denature the tem-plate.
4. Perform 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 5 sec

   Anneal: 45 - 72°C for 15 sec

   Extend: 72°C for 10 sec/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.