GENTAUR

DATA SHEET

Bestaq DNA Polymerase

Store at -20°C

Cat. No.	Description	Concentration	Quantity
G456	Bestaq DNA Polymerase	5 U/µl	250 U
G457	Bestaq DNA Polymerase	5 U/µl	1000 U

Product Description
Bestaq DNA polymerase is a newly engineered polymerase with superior fidelity and robustness. Bestaq's unique, single-enzyme structure results in enhanced processivity, yield, speed, amplification length and tolerates even the most difficult templates. This enzyme is the ideal choice for any PCR application and is the perfect choice for cloning difficult, both AT- and GC-rich, or long amplicons. With one of the highest fidelities available on the market, over 50X better than Taq DNA polymerase, Bestaq DNA polymerase will consolidate all PCR protocols and reactions into one efficient system. This thermostable enzyme generates blunt-end products and has 5'-3' polymerase activity and 3'-5' proofreading exonuclease activity.

Product Components	250 U	1000 U
Bestaq 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
5X GC Enhancer	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.

Shipping and Storage
Upon arrival, Bestaq DNA Polymerase should be stored at -20°C. Avoid repeated freeze-thaw cycles of all Bestaq components to retain maximum performance. All Bestaq 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 (10 µM)	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
Bestaq DNA Polymerase (5 U/µl)	0.5 - 1 µl	2.5 - 5 U
5X GC Enhancer (optional)**	10 µl	1X
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.

** 5X GC Enhancer is recommended for PCR amplification of GC-rich DNA templates.

- *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 template.
4. Perform 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 10 sec

   Anneal: 45 - 72°C for 30 sec

   Extend: 72°C for 1 min/3 - 4 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.

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