Antibody-Based Hot-Start DNA Polymerase

Catalogue No.:abx461028

Abbexa's Antibody-Based Hot-Start DNA Polymerase is a novel PCR enzyme specifically designed for TA cloning, amplifying genomic DNA up to 10 kb and offering 3.5 times higher fidelity than native Taq. Its antibody-based hot-start property allows reaction set-up at room temperature, eliminating all non-specific priming and the formation of primer-dimers. A 5X Reaction Buffer is also included, containing a proprietary mix of dNTPs, $MgCl_2$ and enhancers at optimal concentrations.

Contents:

Component	250 U	500 U	2500 U
Antibody-Based Hot-Start DNA Polymerase	125 µl	250 µl	2 × 625 µl
5X Reaction Buffer	625 µl	1.25 ml	5 × 1.25 ml

Target: Antibody-Based Hot-Start DNA Polymerase

Tested Applications: PCR

Form: Liquid

Biological Activity: One unit is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

Storage: Store all components at -20°C. It is not recommended to store the enzyme at -80°C as ice crystals may form on the active site, which can affect the enzyme activity. Avoid repeated freeze/thaw cycles.

Validity: Up to 12 months.

Buffer: 5X Reaction Buffer: 1 mM dNTPs, 3 mM $MgCl_2$, and enhancers.

Concentration: 2 U/µl

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Reaction Components:

Component	Volume
Template	Variable, as required
Primers (20 µM)	0.5 µl
5X Reaction Buffer	5 µl
Antibody-Based Hot-Start DNA Polymerase	1 µl
Water	Variable, up to 25 µl
Total Volume	**25 µl**

Thermal Cycling Conditions:

Step	Number of Cycles	Temperature	Time per Cycle
Initial Denaturation	1 cycle	95 °C	1 min
Denaturation	25-35 cycles	95 °C	15 seconds
Annealing		≥ 55 °C (primer dependent)	15 seconds
Extension		72 °C	15 seconds

Notes:

• The 5X Reaction Buffer contains 1 mM dNTPs, 3 mM $MgCl_2$ and enhancers. The concentration of each component has been extensively optimized and should only be adjusted if necessary. Additional dNTPs and PCR enhancers such as DMSO are not recommended and may lead to PCR failure.

• Forward and reverse primers are generally used at a final concentration of 0.2-0.6 µM each. It is recommended to start with 0.4 µM as the final concentration (i.e. 10 pmol of each primer per 25 µl reaction volume). A primer concentration that is too high can reduce the specificity of priming, resulting in non-specific products. Primers should have a melting temperature (T_m) of approximately 60 °C.

• The amount of template in the reaction depends mainly on the type of DNA used. For templates with low structural complexity, such as plasmid DNA, it is recommended to use 25 pg - 5 ng DNA per 25 µl reaction volume. For eukaryotic genomic DNA, it is recommended to use a starting amount of 100 ng DNA per 25 µl reaction, this can be varied between 2.5 ng - 250 ng. It is important to avoid using templates re-suspended in EDTA-containing solutions (e.g. TE buffer) since EDTA chelates free Mg^{2+}.

• The initial denaturation step at 95 °C for 1 minute is required to activate the enzyme and fully melt the template. For most PCR, this is sufficient to melt the DNA template, though complex templates such as eukaryotic genomic DNA may require up to 3 min.

• It is recommended to run the subsequent denaturation steps at 95 °C for 15 seconds each cycle, which is suitable for GC-rich templates. Increasing this step up to 30 seconds may improve problematic reactions.

• The optimal annealing temperature is primer dependent and is usually 2-5 °C below the lower T_m of the pair. It is recommended to start with an annealing temperature of 55 °C and, if necessary, run a temperature gradient to determine the optimal annealing temperature. 15 seconds per cycle is usually sufficient, though increasing this step up to 45 seconds may improve problematic reactions.

• The optimal extension time is dependent on the length of the amplicon and the complexity of the template. An extension time of 15 seconds is sufficient for amplicons under 1 kb. Longer extension times are recommended for fragments larger than 1 kb. The extension time may be increased up to 45 seconds/kb to find the fastest optimal condition.

• The optimal conditions will vary from reaction to reaction and are dependent on the system used. Each parameter needs to be adjusted by the end user and some optimization may be required.

• It is recommended to use at least 1 µl of Heat-Activated DNA Polymerase per 25 µl reaction volume.

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