Antibody-Based Hot-Start Taq DNA Polymerase

Catalogue No.:abx461039

Abbexa's Antibody-Based Hot-Start Taq DNA Polymerase is a high performance enzyme designed for fast and highly-specific PCR. 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
Taq DNA Polymerase	50 µl	200 µl	2 × 250 µl
5X Reaction Buffer	2 × l ml	8 × 1 ml	14 × 1.5 ml

Target: Antibody-Based Hot-Start Taq DNA Polymerase

Tested Applications: PCR

Form: Liquid

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: 5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers.

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.

Concentration: 5 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)	1 µl
5X Reaction Buffer	10 µl
Antibody-Based Hot-Start Taq DNA Polymerase	1 µl
Water	Variable, up to 50 µl
Total Volume	**50 µl**

Colony PCR:

Antibody-Based Hot-Start Taq DNA Polymerase can be used for amplification of plasmid DNA directly from liquid cultures or from colonies on agar plates.

• **Liquid Culture:** Up to 8 µl of overnight culture can be added directly to the final reaction mix.

• **Colonies on Agar Plates:** Use a sterile tip to swab the colony and resuspend directly in the 50 µl reaction mix.

Thermal Cycling Conditions (Multiplex PCR of fragments up to 1 kb):

Step	Number of Cycles	Temperature	Time per Cycle
Initial Denaturation	1 cycle	95 °C	1 min
Denaturation		95 °C	15 seconds
Annealing	25-35 cycles	≥ 55 °C (primer dependent)	15 seconds
Extension		72 °C	90 seconds

Notes:

• The 5X Reaction Buffer contains 5 mM dNTPs, 15 mM $MgCl_2$, stabilizers 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 Betaine 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. 20 pmol of each primer per 50 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 50 pg - 10 ng DNA per 50 µl reaction volume. For eukaryotic genomic DNA, it is recommended to use a starting amount of 100 ng DNA per 50 µl reaction, this can be varied between 5 ng - 500 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 recommended to fully melt non-complex templates such as plasmid DNA or cDNA. Complex templates such as eukaryotic genomic DNA may require up to 3 min to completely melt.

• It is recommended to run the subsequent denaturation steps at 95 °C for 15 seconds each cycle, which is suitable for GC-rich templates. For low GC content (40-45%) templates, the denaturation time can be decreased to 5 seconds.

• 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. The annealing time may be reduced to 5 seconds depending on the reaction.

• The optimal extension time is dependent on the length of the amplicon and the complexity of the template. An extension time of 10 seconds is sufficient for low complexity templates such as plasmid DNA. Longer extension times are recommended for fragments larger than 1 kb (e.g. eukaryotic DNA). The extension time may be increased up to 30 seconds/kb to find the fastest optimal condition. Multiplex PCR requires a longer extension step; it is recommended to start with a minimum of 90 seconds and increase if required.

• 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.

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