GB Taq DNA Polymerase **For Research use only**

Cat No: GB2410

Size : 500 unite Concentration : 5u/ul

Description:

GB Taq DNA Polymerase is a thermostable recombinant DNA polymerase resulting from thermophilic bacterium Thermus aquaticus. GB Taq DNA Polymerase can amplify DNA target up to 5 kb (simple template). Its molecular weight is 94 kDa. The elongation velocity is 0.9~1.2kb/min (70~75°C). It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product.

How to use:

General protocol
Components for a 50 µL Reaction

Component	Volume (µL)	Final Concentration
10X GB Standard Taq Buffer	5	1x
10 mM dNTPs	1	200 µM
Forward Primer	1	0.2 µM
Reverse Primer	1	0.2 µM
Template DNA	variable	<1,000 ng
GB Taq DNA Polymerase(GB2410)	0.25	1.25 units/50 µL
Nuclease-free water	Up to 50	

Specific components and steps of the PCR protocol with GB Taq DNA polymerase:

Initial Denaturation

This step is crucial as it ensures that the double-stranded DNA template is fully denatured into single strands, which are accessible for primer binding. Typically performed at 95°C for 30 seconds, this step can be extended for templates that are rich in GC content or particularly complex.

Primers

- **Forward Primer and Reverse Primer**: These are short sequences of nucleotides that are designed to anneal to the target DNA sequence. They define the region of DNA to be amplified.
- **Concentration**: Typically used at a final concentration of 0.2 µM each. Primer design is critical for specificity and efficiency of the PCR.

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dNTPs (Cat No: GB2412)

- **Deoxynucleotide Triphosphates (dNTPs)**: These are the building blocks that Taq DNA polymerase uses to synthesize new DNA strands. A common final concentration is 200 µM each.

Template DNA

- **DNA Template**: This is the sample DNA containing the sequence you wish to amplify. The amount can vary, but generally, less than 1,000 ng is used to avoid inhibition of the reaction.

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- **Enzyme Concentration**: Typically, 1.25 units per 50 µL reaction is sufficient. Overuse can lead to nonspecific amplification and artifacts.

Thermal Cycling Steps

1. **Denaturation**: High temperatures (95°C) to melt the double-stranded DNA.
2. **Annealing**: Lower temperatures (45-68°C) to allow primers to bind to the single-stranded DNA. The exact temperature depends on the primers' melting temperatures.
3. **Extension**: Intermediate temperature (68°C) for Taq polymerase to synthesize the new DNA strand. The duration depends on the length of the target sequence, generally 1 minute per kb.

Final Extension

- **Final Extension**: This step ensures that any remaining single-stranded DNA is fully extended. Typically performed at 68°C for 5 minutes.

Hold

- **Hold**: The reaction is kept at a low temperature (4-10°C) to preserve the amplified DNA until it can be analyzed.

Optimization Tips

- **Magnesium Concentration**: Mg^{2+} is a critical cofactor for Taq DNA polymerase. Adjusting the concentration (1.5-2.0 mM) can improve the efficiency and specificity of the PCR.
- **Additives**: For difficult templates, additives like DMSO or formamide can help by reducing secondary structures and improving denaturation.

Tips

- **Mineral Oil**: If your thermocycler doesn't have a heated lid, overlay the sample with mineral oil to prevent evaporation.
- **Optimization**: Adjust Mg^{2+} concentration (1.5-2.0 mM) and consider additives like DMSO or formamide for difficult targets.
- **Quality of DNA**: Use high-quality, purified DNA templates for better results

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