Tinzyme Co., Limited

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Fast bisDNA Probe Kit

Product Number: PCK32

Shipping and Storage

-20±5°C,try to avoid repeated freeze-thaw cycles as much as possible

Components

Component	PCK32 500U	PCK32 5000U
Fast bisDNA Probe Kit Buffer	2×1.2mL	2×12mL
FastStar DNA Polymerase (5U/μL)	100μL	1mL

Description

This product is mainly used for probe based fluorescence quantitative PCR using DNA treated with bisulfite as a template.Fast Star DNA Polymerase is a novel and highly efficient hot start enzyme modified with dual monoclonal antibodies. The enzyme's activity is completely blocked at room temperature, effectively avoiding non-specific amplification caused by non-specific binding of primers and templates or primer dimers at room temperature. The optimized Fast BisDNA Probe Kit Buffer includes PCR Buffer, dNTPs, and Mg^{2+}, and only requires customers to add templates, primers, and probes, Easy to use.

Notes

1. Before use, please gently mix the product upside down after it has completely melted, and centrifuge briefly before use.
2. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance. This product can be stored at -20℃ for a long time, away from light.If frequent use is required in the short term, it can be stored at 2-8℃.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system

Reagent	25μL reaction system	50μL reaction system	Final Concentration
Fast bisDNA Probe Kit Buffer	14 μL	28 μL	1×
Forward Primer,10 μM	0.5 μL	1 μL	0.2 μM [1)]
Reverse Primer,10 μM	0.5 μL	1 μL	0.2 μM[1)]
Probe,10 μM	0.5 μL	1 μL	0. 2 μM[2)]
FastStar DNA Polymerase	0.6 μL	1.2 μL	
Template DNA	X μL	X μL	
ddH_2O	Up to 25 μL	Up to 50 μL	

Note:1)Typically, the primer concentration is 0.2μM can achieve good results, ranging from 0.1-1.0μM serves as a reference for setting the range.

2)The concentration of the probe used is related to the fluorescent quantitative PCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

3)Usually, the amount of DNA templates is based on 10-100ng genomic DNA or 1-10ng cDNA. Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be performed on the templates to determine the optimal template usage.

2. PCR reaction condition

Step	Temperature	Time	cycles
Predenaturation	95℃	30s[1)]	1
Denaturation	95℃	10s	45 -45
Annealing/Extend	60℃	30s[2)]	

Note:1)The initial denaturation of this product at 95℃ for 30 seconds is sufficient to activate the enzyme; complex templates can be extended to 3 minutes for denaturation

2)It is recommended to use a two-step PCR reaction program.If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted.The annealing temperature should be set within the range of 56℃-64℃ as a reference.