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Version: 09/2023

FastStar Probe Kit (for bisDNA)

Cat. No. : CW3332S (500 U)
CW3332M (5000 U)

Shipping and Storage : Storage at -20±5°C, avoid repeated freeze-thaw.

Components

Component	CW3332S 500 U	CW3332M 5000 U
FastStar Probe Buffer (for bisDNA)	2×1.2 mL	2×12 mL
SuperFastStar DNA Polymerase (5U/μL)	100 μL	1 mL

Principle

This product is mainly used in quantitative fluorescence PCR by probe method using bisulfite treated DNA as template. The SuperFastStar DNA Polymerase is a new type of hot start enzyme modified by double monoclonal antibody. The enzyme activity is completely blocked at room temperature, which effectively avoids the non-specific amplification caused by the nonspecific binding of primer and template or primer dimer at room temperature. The optimized FastStar Probe Buffer (for bisDNA) includes PCR Buffer, dNTPs, Mg^{2+}, etc. It only needs to be added with templates, primers and probes, which is easy to use.

Note

1. After the product is completely melted, mix it gently upside down and centrifuge briefly before use.
2. Avoid repeated freeze-thaw, which may degrade product performance. This product can be stored at -20°C for a long time. This product needs to be stored away from light. If you need to use frequently in the short term, it can be stored at 2-8°C.

Procedure

The following examples are the conventional PCR reaction system and reaction conditions, which should be improved and optimized according to different template, primer structure and target fragment size in actual operation.

1. PCR reaction system

Reagent	25 μL system	50 μL system	Final concentration
FastStar Probe Buffer (for bisDNA)	14 μL	28 μL	1 ×
Forward Primer, 10μM	0.5 μL	1 μL	0.2μM[1)]
Reverse Primer, 10μM	0.5 μL	1 μL	0.2μM[1)]
Probe, 10 μM	0.5 μL	1 μL	0.2μM[2)]
SuperFastStar 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) Generally, a primer concentration of 0.2μM can get better results, and 0.1-1.0μM can be used as a reference for the set range.
2) The concentration of the probe to use is related to the fluorescence quantitative PCR instrument, the type of probe and the type of fluorescent labeled substance. Please adjust the concentration according to the instructions of the instrument or the specific requirements for the use of each fluorescent probe.
3) The amount of DNA template is usually 10-100 ng genomic DNA or 1-10 ng cDNA as reference. Due to the different number of target gene copies contained in the template of different species, gradient dilution can be carried out on the template to determine the best use of template.

2. PCR reaction conditions

Step	Temperature	Time	Cycle
Predenaturation	95°C	30 s[1)]	1
Denaturation	95°C	15 s	40-45
Annealing/extension	60°C	30 s[2)]	40-45

Note:
1) Initial denaturation of the product at 95°C for 30s is sufficient to activate the enzyme. Denaturation of complex templates can be extended to 3min.
2) It is recommended to adopt the two-step PCR reaction procedure. If the results are not good due to the use of primers with low Tm value, the three-step PCR amplification can be attempted. Please set the annealing temperature in the range of 56°C to 64°C as a reference.