BioSmart U⁺ Taq HS DNA Polymerase

P142

Version 24.2

Product Description

BioSmart U⁺ Taq HS DNA Polymerase is an enzyme selected through the BioSmart platform. It has a relatively long sequence of shortest extended bases, strong 3' mismatch recognition ability and high specificity. As the hot-start Taq DNA polymerase, BioSmart Taq HS DNA Polymerase contains high blocking rate dual-species antibodies that are able to maintain strict blocking at 55°C by binding different antigenic epitopes. With the optimized buffer system, this product effectively reduces non-specific amplification caused by primer-probe mixes, during sample mixing, system temperature increasing, and long-term storage, thus supporting the premixed format of primers and probes. When the reaction is maintained at 95°C for more than 30 sec, the antibody is completely inactivated, and the Taq enzyme activity is fully released, ensuring the sensitivity and specificity of PCR amplification. This product is in the format of enzyme and buffer separation, thereby the concentrations of enzyme, Mg^{2+}, and dNTP can be flexibly adjusted according to the differences of various systems. It is applicable for various PCR and qPCR based on the hot-start Taq enzyme. In addition, this product provides heat-labile UDG, dUTP, and dTTP to establish an anti-contamination system, thereby minimizing PCR cross-contamination. Users can optimize and adjust according to actual requirements to get better experimental results.

Components

Components	P142-d1 200 rxns (20 μl/rxn)	P142-d2 500 rxns (20 μl/rxn)
BioSmart Taq HS DNA Polymerase (5 U/μl)	80 μl	200 μl
10 × BioSmart Taq HS Buffer (Mg^{2+} free)	1 ml	2 × 1.25 ml
$MgCl_2$ (100 mM)	200 μl	500 μl
dA/C/GTP Mix (10 mM each)	200 μl	500 μl
dTTP (25 mM)	100 μl	250 μl
dUTP (25 mM)	100 μl	250 μl
Heat-labile UDG (2 U/μl)	40 μl	100 μl

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for the amplification and quantification of DNA samples from all species sources. The type of sample can be genomic DNA, plasmid DNA, etc.

Source

Taq DNA Polymerase is expressed from *E. coli* that contains the *Thermus aquaticus* DNA Polymerase gene.

Unit definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble material in 30 min at 74°C, with activated salmon sperm DNA as the template/primer.

Notes

Primer Design Guidance

1. The ideal primer length is 17 - 25 bp. Primers that are too short are likely to result in reduced amplification efficiency. Primers that are too long are more likely to generate higher structures. Both of them will interfere with the accuracy of quantitative results.
2. Control the GC content of the primers at 40% - 60%, and the optimum GC content is from 45% - 55%.
3. The Tm value of the primer should be greater than 60°C. Primer Premier 5 is recommended to calculate the Tm value.
4. The overall distribution of A, G, C, and T in the primer should be as uniform as possible. Avoid using regions with high GC or TA content, especially at the 3' end. Regions with uneven GC content must be avoided.
5. Try to avoid structures with consecutive T/C or A/G when designing primers.
6. The last five bases at the 3' end of the primer must not contain more than two G or C.
7. Ensure that the forward or the reverse primer is as close as possible to the probe sequence, but does not have overlap regions with the probe sequence.

TaqMan Probe Design Guidance

1. The probe sequence should be as close as possible to the forward or reverse primer, but overlap sequences must be avoided.
2. The probe length is typically 18 - 40 bp.
3. Avoid consecutive identical bases, especially four or more consecutive G.
4. Avoid using G at the 5' end of the probe.
5. The annealing temperature of the probe should be 65 ~ 67°C.
6. If there is polymorphic locus in the sequence, make it lie in the middle of the probe.

Experiment Process (Using ABI QuantStudio 5 as a test machine)

1. Prepare the following mixture in a qPCR tube

Components	Volume	
10 × BioSmart Taq HS Buffer (Mg^{2+} free)	2 μl	
BioSmart Taq HS DNA Polymerase (5 U/μl)[a]	0.4 μl	
$MgCl_2$ (100 mM)[b]	0.3 - 1 μl	
dA/C/GTP Mix (10 mM each)	0.4 μl	
dTTP (25 mM)	0.08 μl	
dUTP (25 mM)	0.08 μl	
Heat-labile UDG (2 U/μl)	0.1 μl	
Primer Mix	X μl	
TaqMan Probe Mix	Y μl	
Template DNA	Z μl	
ddH_2O	Up to 20 μl	

The volume of each component in the reaction system can be adjusted according to the following principles:

a. The amount of enzyme can be adjusted between 0.25 - 1 μl. Increasing the amount of enzyme will normally increase the amplification yield, but the specificity may be reduced.

b. The concentration of Mg^{2+} can be adjusted between 1.5 - 5 mM. Increasing the concentration of Mg^{2+} will normally increase the amplification yield, but the specificity may be reduced.

▲ Generally, a good result can be obtained when the final concentration of primer in the reaction system is 0.2 μM. If the result is not as expected, the primer concentration can be adjusted between 0.1 - 1.0 μM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of experiments, it is recommended to dilute the template and add it to the reaction system.

▲ The volume of templates can be adjusted appropriately.

2. Perform qPCR reaction under the following conditions

Stage 1	Contamination Digestion	Rep: 1	37°C	2 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 45	95°C	10 sec
			60°C	30 sec*

* For signal acquisition.