TIANGEN®

High Affinity HotStart *Taq*

High affinity antibody-modified HotStart DNA polymerase

www.tiangen.com/en

High Affinity HotStart *Taq*

Cat. No. 4992773/4992774

Kit Contents

Contents	4992773	4992774
High Affinity HotStart *Taq* (5 U/μl)	250 U	500 U
10×HA Buffer	1.8 ml	1.8 ml
5×Probe qPCR Buffer	1 ml	2 × 1 ml
Handbook	1	1

Storage Conditions

The product can be stored at -30~-15°C for one year. Please store the product at -30~-15°C immediately after receiving it. The product can be kept for 1 year at -30~-15°C. When taken out from -30~-15°C, thaw the frozen 10 × HA buffer and 5 x Probe qPCR Buffer, then shake upside down slightly to mix. Apply the solutions when the solution is completely homogeneous. If the buffers are used frequently for a period of time, they can be stored at 2-8°C for 3 months. Repeated freezing and thawing should be avoided.

Product Description

The High Affinity HotStart *Taq* in this product is a mixture of TIANGEN *Taq* DNA Polymerase and a monoclonal antibody to *Taq* DNA polymerase, which is suitable for the HotStart PCR experiment. *Taq* monoclonal antibody will combine with *Taq* polymerase and inhibit its polymerase activity before the PCR temperature is elevated. The high affinity antibody in this product can completely inhibit the enzyme activity of *Taq* polymerase under normal temperature to keep the whole reaction system with high specificity. In addition, the *Taq* DNA polymerase in this product has high template affinity, which can improve the efficiency and specificity of amplification. The 10 × HA Buffer is a specially optimized PCR reaction buffer for this polymerase, which can guarantee the best performance of the enzyme. The 5 × Probe qPCR Buffer is a reaction additive specially optimized for the probe qPCR, which can be used in the probe qPCR reaction in combined with the 10 × HA Buffer, ensuring a wider application of the product. In addition, the 3'-end of the PCR product produced by the kit is A, which can be cloned directly with TA vector.

Product Highlights

1. High affinity system: High Affinity HotStart *Taq* is a specific antibody modified hot start DNA polymerase with high antibody affinity. The *Taq* DNA polymerase has high template affinity, stable amplification efficiency and high specificity, and the well-designed buffer system also makes the PCR reaction with high sensitivity.
2. Strong stability: For complex templates, low copy templates, multiplex PCR reaction and other experiments that ordinary DNA polymerase cannot perform, but this product has a high reaction success rate.
3. Wide application: This product is not only suitable for general PCR analysis, but also for quantitative PCR analysis.

Precautions

1. There is no need to add the 5 × Probe qPCR Buffer in general PCR and dye-based quantitative PCR, but only in probe quantitative PCR.
2. In probe quantitative PCR, 250 nM final concentration of primers and 200 nM final concentration of probe can lead to good amplification results in most systems. If the primer concentration needs to be further optimized, it can be adjusted in the range of 50-900 nM. If the probe concentration needs to be remove several nucleotides from 5' or 3' terminal.

Definition of activity

The activity of 1 unit (U) High Affinity HotStart *Taq* is defined as the amount of enzyme required to mix 10 nmol of deoxynucleotides into acid insoluble substrates in 3 minutes at 74°C with the active sperm DNA of salmon as template/primers.

Quality Control

The purity of SDS-PAGE is more than 99%; No activity of exogenous nuclease is detected; Single gene in human genome could be amplified effectively; No significant activity change when stored at room temperature for one week.

Applications

The product is suitable for conventional PCR reaction, complex template, low copy template amplification, multiplex PCR and quantitative PCR, etc.

Protocol

<1> Routine PCR reaction:

Note: The following examples are for reference only. The actual reaction conditions are different due to the different structures of templates and primers. The best reaction conditions should be set according to the actual situation.

1. Use High Affinity HotStart Taq as the polymerase, and human genome DNA as template to amplify 1000 bp fragment.
2. Prepare the reaction solution according to the table below:

Component	50 µl reaction	20 µl reaction	Final concentration
DNA Template	–	–	< 200 ng
dNTPs (2.5 mM each)	4.0 µl	1.6 µl	200 µM
Forward primer (10 µM)	1.25 µl	0.5 µl	250 nM
Reverse primer (10 µM)	1.25 µl	0.5 µl	250 nM
10×HA Buffer	5.0 µl	2.0 µl	1×
High Affinity HotStart *Taq* (5 U/ µl)	0.5 µl	0.2 µl	0.05 U/ µl
RNase-Free ddH_2O	Up to 50 µl	Up to 20 µl	–

3. Set up the PCR program according to the table below:

Step	No. of cycles	Temperature	Time	Details
Initial denaturation	1×	95°C	3-5 min	Initial denaturation
PCR cycling	35-40×	94°C	15 sec	Denaturation
		60°C	20 sec	Annealing
		72°C	1 min	Extension
Final extension	1×	72°C	5 min	Extension

4. Results: Once the program is completed, load 10 µl to agarose gel electrophoresis to detect the DNA products.

   Note: the results show that the repeated freezing and thawing of DNA template will affect the amplification efficiency, so avoid freeze and thaw the DNA template repeatedly. If the template needs to be applied multiple times, aliquot it first before store at -20°C to reduce freezing and thawing times.

< 2 > Dye-based Real-Time PCR reaction system:

1. Thaw the reagents required for the reaction, balance to room temperature and mix them thoroughly.
2. It is recommended to prepare the Real-Time PCR reaction solution on ice. Prepare the reaction solution according to the table below:

Component	50 µl reaction	20 µl reaction	Final concentration
Template	–	–	–*1
dNTPs (2.5 mM each)	4.0 µl	1.6 µl	200 µM
Forward primer (10 µM)	1.25 µl	0.5 µl	250 nM*2
Reverse primer (10 µM)	1.25 µl	0.5 µl	250 nM*2
10× HA Buffer	5.0 µl	2.0 µl	1×
High Affinity Hot start *Taq* (5 U/ µl)	0.5 µl	0.2 µl	0.05 U/ µl
20×SYBR Solution	2.5 µl	1.0 µl	1×
50×ROX Reference Dye*3	–	–	–
RNase-Free ddH_2O	Up to 50 µl	Up to 20 µl	–

*1 When the template is genomic DNA, the amount of template is 50-100 ng, when the template is cDNA, the amount of template is no more than 1/10 of PCR reaction system.

*2 The final concentration of the primers is 250 nM, which can lead to good amplification results in most systems. The primer concentration in the PCR reaction system can be increased If the amplification efficiency is not high. When non-specific amplification occurs, the primer concentration in the PCR reaction system can be appropriately reduced. if the primer concentration needs to be further optimized, it can be adjusted in the range of 50-900 nM.

*3 The optimal ROX Reference Dye concentration for several common instruments is shown in the table below:

Instrument	Final concentration
ABI PRISM 7000/7300/7700/7900HT/Step One etc.	2.5×(example:2.5 µl ROX/50 µl system)
ABI 7500, 7500 Fast, Stratagene Mx3000P, Mx3005P, Mx4000, etc.	0.5× (example:0.5 µl ROX/50 µl system)
Roche, Bio-Rad, Eppendorf Instruments, etc.	NA

3. Set up the PCR program according to the following table:

Step	No. of cycles	Temperature	Time	Details	Fluorescent signal acquisition
Initial denaturation	1×	95°C	3-5 min	Initial denaturation	No
PCR cycling	40-45×	95°C	15 sec	Denaturation	No
		60°C	30 sec	Annealing/ extension	Yes
Melting curve	1×	65-95°C	–	Melting curve	Yes

4. Cover the reaction tube and mix gently. Pulse-spin the tube to ensure that all the reaction liquid is at the bottom of the tube.
5. Put the reaction system in the thermal cycler and start the program.
6. Analyze the experimental results.

After the reaction, get the Real Time PCR amplification curve and melting curve, and make the standard curve for PCR quantification.

<3> Probe Real Time PCR reaction system:

1. Thaw the reagents required for the reaction, balance to room temperature and mix them thoroughly.
2. It is recommended to prepare the Real-Time PCR reaction solution on ice. Prepare the reaction solution according to the table below:

Component	50 μl reaction	20 μl reaction	Final concentration
Template	–	–	–[*1]
dNTPs (2.5 mM each)	4.0 μl	1.6 μl	200 μM
Forward primer (10 μM)	1.25 μl	0.5 μl	250 nM[*2]
Reverse primer (10 μM)	1.25 μl	0.5 μl	250 nM[*2]
Fluorescent probe (10 μM)	1.0 μl	0.4 μl	200 nM[*3]
10× HA Buffer	5.0 μl	2.0 μl	1×
5×Probe qPCR Buffer	10 μl	4.0 μl	1×
High Affinity HotStart *Taq* (5 U/ μl)	0.5 μl	0.2 μl	0.05 U/ μl
50×ROX Reference Dye[*4]	–	–	–
RNase-Free ddH_2O	Up to 50 μl	Up to 20 μl	–

[*1] When the template is genomic DNA, the amount of template is 50-100 ng, when the template is cDNA, the amount of template is no more than 1/10 of PCR reaction system.

[*2] The final concentration of the primers is 250 nM, which can lead to good amplification results in most systems. The primer concentration in the PCR reaction system can be increased If the amplification efficiency is not high. When non-specific amplification occurs, the primer concentration in the PCR reaction system can be appropriately reduced. If the primer concentration needs to be further optimized, it can be adjusted in the range of 50-900 nM.

[*3] The concentration of the probe is related to the Real Time PCR thermal cycler, probe type and fluorescent labeling substance type used. Please refer to the instructions of the instrument or the specific instructions of each fluorescent probe for specific use. In general, a probe with a final concentration of 200 nM can obtain good amplification results in most systems. If the probe concentration needs to be further optimized, it can be adjusted in the range of 100-500 nM.

[*4] For the use of Rox reference dye, please refer to the dye-based Real Time PCR reaction system.

3. Real-time PCR reaction

It is recommended to use the two-step PCR procedure. The denaturing time can be adjusted in the range of 5-15 sec, and the annealing/extension time can be adjusted in the range of 20-32 sec.

Two-step PCR program:

Step	No. of cycles	Temperature	Time	Details	Fluorescent signal acquisition
Initial denaturation	1×	95°C	3-5 min	Initial denaturation	No
PCR cycling	40-45×	95°C	5-15 sec[*1]	Denaturation	No
		60°C	15-32 sec[*2]	Annealing/extension	Yes

[*1] To set the time on different types of instruments, please refer to the requirements of the instrument operation manual. When using ABI 7900HT/7900HT Fast/Viia 7/StepOne/StepOnePlus, it can be set to 5 sec.

[*2] To set the time on different types of instruments, please refer to the requirements of the instrument operation manual.

The time setting of several common instruments is shown in the table below:

For ABI 7900HT/7900HT Fast/ViiA 7/StepOne/StepOnePlus, set 20 sec
For Roche LightCycler/LightCycler 480, ABI 7500 Fast, set 15 sec
For ABI 7000 and 7300, set 31 sec
For ABI 7500, set 32 sec

4. Cover the reaction tube and mix gently. Pulse-spin the tube to ensure that all the reaction liquid is at the bottom of the tube.
5. Put the reaction system in the thermal cycler and start the program.
6. Analyze the experimental results.