Instruction manual anti-Taq high 1108

anti-Taq high

TCP-101, 100 µl
(Correspond to 500 U Taq DNA polymerase)
Store at -20 °C.

Contents

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions and follow safety guidelines while using this kit.

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[1] Introduction

Description

anti-Taq high is a highly purified neutralization monoclonal antibody to Taq DNA polymerase. This product provides an antibody-mediated hot start PCR to enhance the specificity and sensitivity of PCR. This antibody inhibits polymerase activity before the onset of thermal cycling, preventing primer dimer formation and non-specific amplification. At the first denaturation step of the thermal cycling, the antibody is quickly inactivated and PCR proceeds. The antibody-mediated hot start method is significantly more convenient to use than other hot start methods.

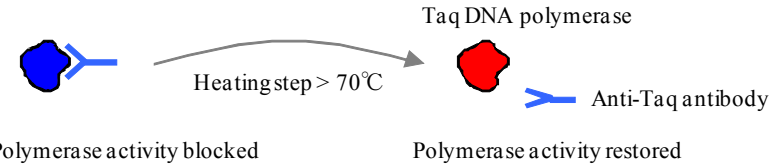

Fig. 1. Principle of Blunting by KOD DNA polymerase

Features

- Inhibits ≥95% polymerase activity at 45ºC
-No contamination of mouse genomic DNA examined by PCR
-Enhances the specificity and sensitivity of PCR
-Can be used when other hot start methods are difficult to perform (*e.g.* capillary PCR)
-The polymerase can be reactivated quicker than with methods utilizing a chemically modified polymerase

[2] Components

anti-Taq high*	100 µl
10 x PCR Buffer**	1 ml

*100 µl of anti-Taq high; corresponds to 500 U Taq DNA polymerase.
** <10 x PCR Buffer >
10 mM Tris-HCl(pH8.3), 500 mM KCl, 15 mM $MgCl_2$
**PCR Buffers supplied with purchased Taq DNA polymerases can be used instead of this buffer.

[3] Protocol

1.Preparation of polymerase-antibody mixture

{ Taq DNA polymerase* 1 U
+ anti-Taq high 0.2 µl } → Room temperature, 5 min → Use as a polymerase solution**

or

{ Taq DNA polymerase* (5 U/µl)
+ Equal volume of anti-Taq high }

*Taq-based high efficient PCR enzymes can be used.
**The polymerase-antibody mixture can be stored for long term at -20 °C.

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2. Typical reaction set up

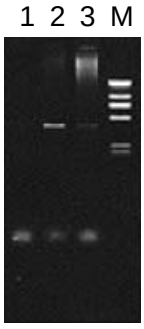
Component	Volume
Distilled water	37.2-X µl
10 X PCR buffer	5 µl
2 mM dNTPs	5 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
Polymerase-antibody mixture (2.5U/µl)	0.8 µl
Template DNA	X µl
Total Volume	50 µl

Note: Pre-denaturation step is not necessary on PCR cycling.

[4] Example

Example 1. Application of the hot start PCR using the Taq based high efficient PCR enzyme

The efficiency of anti-Taq antibodies were evaluated by the amplification of the human β-globin gene (3.6 kb). The result indicates that anti-Taq high increases the specificity and sensitivity of the PCR compared with the control reaction and PCR mediated hot start using company A's anti-Taq antibody.



M: λ/Hind III Marker
1: Taq-based high efficient PCR enzyme
2: Taq-based high efficient PCR enzyme + anti-Taq high
3: Taq-based high efficient PCR enzyme + anti-Taq antibody (company A)

[5] References

1) R.T. D'Aquila, L.J. Bechtel, J.A. Videler, Eron JJ, P. Gorczyca, J.C.Kaplan, Maximizing sensitivity and specificity of PCR by pre-amplification heating. *Nucleic Acids Res*. 19: 3749 (1991)

2) Q. Chou, M. Russell, D.E. Birch, J. Raymond, W. Bloch. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res*. 20: 1717-23 (1992)

3) D.E. Kellogg , I. Rybalkin, S. Chen, N. Mukhamedova, T. Vlasik, P.D. Siebert, A. Chenchik. TaqStart Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *Biotechniques*. 16: 1134-7 (1994)