Hot Start Taq Antibody
Cat. No. A01849 **Version 08102016**

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I DESCRIPTION

The Hot Start Taq Antibody is a mouse monoclonal antibody that specifically binds to Taq DNA polymerase. When this antibody is bound to Taq, the enzyme is rendered inactive. It provides an antibody-mediated hot start that enhances the specificity and sensitivity of PCR. Inhibition is completely reversed when the temperature is raised during the first denaturing step of thermal cycling.

II KIT CONTENTS

Kit Contents	Quantity	Catalog No.	Components/Concentration
Hot Start Taq Antibody	250 U	A01849-250	5 U/μl
	1000 U	A01849-1000	5 U/μl
1×Dilution Buffer	1ml		8.5 g/L NaCl, 1.4 g/L Na_2HPO_4, 0.2 g/L NaH_2PO_4(pH 7.4)

III APPLICATIONS

- **Hot Start PCR**
- **Western Blot Assay**

IV QUALITY CONTROL ANALYSIS

- **Inhibition Assay:** Greater than 95% inhibition is observed after a 30 minute incubation at 65°C.
- **Heat inactivation Assay:** Greater than 95% activity of Taq DNA polymerase is reversed after a 30 second incubation at 95°C.
- **RNase activity assay:** Incubation of a 10 μl reaction containing 10 units of Taq Antibody with 1 μg total RNA for 1 hour at 37°C doesn't result in the degradation of total RNA as determined by agarose gel electrophoresis.
- **DNase activity assay:** Incubation of a 25 μl reaction containing 10 units of Taq Antibody with 500 ng DNA for 16 hours at 37°C doesn't result in the degradation of DNA as determined by agarose gel electrophoresis.

860 Centennial Ave., Piscataway, NJ 08854, USA
Toll-Free: 1-877-436-7274 Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: product@genscript.com Web: www.genscript.com

V UNIT DEFINITION

One unit is defined as the amount of antibody required to inhibit greater than 90% one unit of Taq DNA polymerase after a 10 minute incubation at 55°C.

VI STORAGE

Hot Start Taq Antibody is supplied with 1 X storage buffer (8.5 g/L NaCl, 1.4 g/L Na_2HPO_4, 0.2 g/L NaH_2PO_4 (pH 7.4)). The recommended storage temperature is -20°C. Aliquot to avoid repeated freeze/thaw cycles.

VII ACTIVITY TEST

1. To test the blocking activity of Hot Start Taq antibody, a primer extension assay was done as follows. A pair of primers was designed with a 15 bp overlap. One primer was 24 bp; the other one was 41 bp. The primers were annealed and incubated with taq DNA polymerase with or without antibody for 1 h at 37°C. The extension result was monitored by denature PAGE gel.

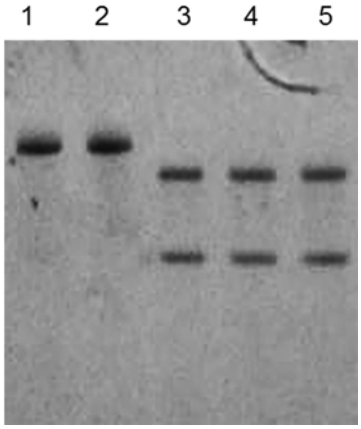

Figure 1. The primer extension assay to detection the blocking activity of Hot Start Taq Antibody. *All groups were added with 1.25 U Taq DNA polymerase pretreated with 0, 0.25, 0.5, 1, 2 U (Lane 1-5) Hot Start Taq Antibody.*

2. To test the specificity of Taq DNA polymerase bounding to hot start taq antibody, the taq:antibody mixture was used in genome amplification assay. The target PCR fragment is HPRT site with 500 bp. Some non-specific bands were observed without hot start taq antibody, while the addition of this antibody improved the specificity obviously.

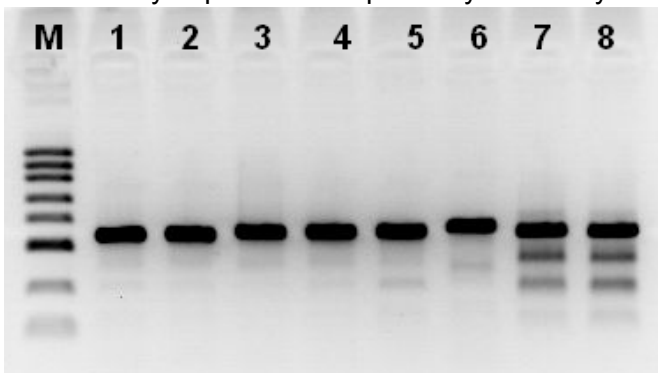

Figure 2. The PCR specificity detection of Hot Start Taq Antibody. *The Antibody used in each group is 2, 1, 0.5, 0 U (lane 1-2, 3-4, 5-6, 7-8).*

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VIII PROTOCOL

1. Prepare a mixture of Taq DNA polymerase and Taq antibody for Hot start PCR.
   Add one volume of Hot Start Taq Antibody to one volume of Taq DNA polymerase. The reagent amounts below are for 20 PCR amplifications.
   5 µl Hot Start Taq Antibody (5 units/µl)
   5 µl Taq DNA polymerase (5 units/µl)
   10 µl Total volume
2. Incubate the mixture for 10 minutes at room temperature.
   Note: This mixture can be scaled up, aliquoted to multiple tubes and stored at –20 °C for up to 6 months.
3. Use the complexed Taq DNA polymerase: Taq Antibody in a standard PCR reaction. Because the concentration of the Taq polymerase has changed, adjust the PCR protocol accordingly. Use the buffer provided with kit or other standard Taq DNA polymerase buffer. For each 50 µl reaction, assemble the following components in a 0.5 ml PCR tube at room temperature:

5 µl	10X Taq Buffer with $MgCl_2$
1 µl	dNTP Mix (10 mM each dATP, dCTP, dGTP, dTTP)
1 µl	5' primer, 10 µM
1 µl	3' primer, 10 µM
0.5 µl (1.25 U)	Taq DNA Polymerase: Taq Antibody Complex
X µl	DNA template (typically 10–100 ng)
Up to 50µl	PCR Grade Water

4. PCR cycling parameters:

Hot Start	5 min	95 °C
Denature	15s	at 94°C
Anneal	15s	at 55-65°C
Extend	1 kb/min	at 72°C
Repeat	for 30 cycles	

   Followed by final extension for 5 min at 72°C.
5. Detection by agarose gel electrophoresis.

Note
For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

GenScript US
860 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
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Email: product@genscript.com
Web: http://www.genscript.com
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