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USER PROTOCOL

OmniStar™ Hot Start Taq Antibody

DESCRIPTION

OmniStar™ is a mouse monoclonal antibody. It blocks polymerase activity during set-up of the PCR reactions. It provides an antibody mediated hot start. OmniStar™ Taq Antibody is effective with the variety of commercial available Taq DNA polymerases (native or recombinant). We recommend to use our OmniBio™ Taq DNA Polymerase.

COMPONENTS

100 µl	Taq Antibody (4 mg/µl; 50 µl is enough to inhibit 1000 units of Taq DNA Polymerase)
1.3 ml	10x PCR Buffer (160 mM(NH ₄) ₂ SO ₄ , 670 mM Tris-HCL at PH 8.8 (at 25°C), 15mM MgCl ₂ , 0,1% Tween 20)

COMMENT

The amount of antibodies required for polymerase activity inhibition depends not on the units of the enzyme, rather on the amount of Taq polymerase as a protein (in mg). The ratio units/mg of Taq Polymerase varies strongly from preparation to preparation (factor of 10 in our tests). In fact, antibodies are good tool to check the amount of "inactive" protein in the specific preparation. We consider 1 mg of our antibodies as 2300 "blocking units", 1 blocking unit is defined as the amount of antibodies required to block 50% activity of 1 µg of Taq DNA polymerase at 37° C. The amount of Taq DNA polymerase units in 1 µg varies from different producers from 5000 units to 50.000 units according to our experience. So, the amount of antibodies for Taq polymerase inhibition will vary correspondingly. As for the exact ratio Taq polymerase / Antibodies, you should be found empirically for the best performance (of course, considering the amount of units required for 50% activity inhibition). We recommend you to try our Omni Star™ Hot Start Taq DNA polymerase - the optimised mixture of Taq DNA polymerase and antibodies.

STANDARD PROTOCOL

In many cases, the standard reaction described below will provide satisfactory amplification. Remember to include a negative control reaction lacking only template; inclusion of a positive control reaction using a template known to amplify with primers may also be helpful. The optimal conditions for the concentrations of enzyme, MgCL₂, template and primers are typically determined empirically.

1. Mix 50 µl of OmniStar™ Hot Start Taq Antibody with 1000 units of OmniBio™ Taq DNA Polymerase or other native or recombinant Taq DNA Polymerase. The concentration of the OmniBio™ Taq DNA polymerase is now 4/µl

Note: The OmniBio™ Taq: Hot Start Taq Antibody complex can be prepared individually for each reaction by combining 1 µl Taq Antibody with 20 units OmniBio™ Taq DNA Polymerase.

2. Incubate for 5 min at 20-25°C. Store the complex 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 DNA Polymerase has changed, adjust the PCR protocol accordingly. Use the buffer provided with the kit or other standard Taq DNA polymerase buffer. For each 50 µl reaction; assemble the following componenets in a 0,5 ml PCR tube at room temperature:

35-37 µl	PCR Grade Water
1 µl	dNTP mix (10 mM each dATP, dCTP, dGTP, dTTP)
1 µl	5' primer, ~5 pmol/µl
1 µl	3' primer, ~5 pmol/µl
5 µl	10x OmniBio™ Taq Buffer without MgCL ₂
3-5 µl	25 mM MgCL ₂
0,5 µl	2 units OmniBlo™ Taq DNA Polymerase: Taq Antibody Complex
1 µl	DNA template (typically 10-100 ng)
50 µl	total volume

4. mix gently and centrifuge briefly if necessary to bring reaction components to bottom of tube. If necessary, add 3 drops of mineral oil (~65 µl), cap the tubes und place in thermal cyc-ler.

5. PCR cycling parameters:

Denature 1 min at 94°C

Anneal 1 min at 60°C

Extend 2 min at 72°C

repeat for 25-30 cycles

followed by final extention for 5 min at 72°C.

6. To analyse the reaction products, remove a 10 µl sample from beneath the oil overlay and add to appropriate loading buffer. Load and run a 1% agarose gel containing 0,5 µg/ml ethidi-um bromide and visualise the bands under UV illuminaton.