ADVANCED TARGETING SYSTEMS

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Anti-Staphylococcus aureus
RABBIT POLYCLONAL
AB-T161

Example of ELISA protocol:

1. Coating of Staphylococcus aureus antigens (4 µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05 M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 5 g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Staphylococcus aureus antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5 g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 µl of peroxidase-labeled goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l of BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1 M/phosphate 0.2 M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark, after that, we will stop the reaction by the addition of 50 µl of 2 M HCl.
9. The optical density will be measured at 492nm.

Example of Western blot protocol used to test *Pseudomonas aureofasciens*:

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti- Staphylococcus aureus antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1 µg/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50µl H_2O_2 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)
TBS = 20mM Tris base, 0.5M NaCl, pH 7.5
TBS Tween = TBS + 0.05% Tween 20