Advanced Targeting Systems

Anti-Pseudomonas putida
RABBIT POLYCLONAL
AB-T138

Example of ELISA protocol used to test *Pseudomonas putida*:

1. Coating of Pseudomonas putida (4µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed Pseudomonas putida serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200µl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200µl of peroxidase-labeled goat anti-rabbit (Jackson) 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 0.1M/phosphate 0.2M (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 50µl of 2M HCl.
9. The optical density will be measured at 492nm.

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

Membrane Blocking, Antiboby 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 the antibody 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 1:1000 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 1µg/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 H2O2 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