



**Anti-Pseudomonas marginalis
RAT POLYCLONAL
AB-T059**

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

1. Coating of *Pseudomonas marginalis* (4 μ g/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 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 marginalis* serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA, 200 μ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 μ l of peroxidase-labeled goat anti-rat (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 200 μ l 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 50 μ l of 2M HCl.
9. The optical density will be measured at 492nm.

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

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 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 1 μ g/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) + 50 μ l 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