



**Anti-Pseudomonas fluorescens  
RABBIT POLYCLONAL  
AB-T063**

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

1. Coating of *Pseudomonas fluorescens* ( $4\mu\text{g/ml}$ ) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at  $4^{\circ}\text{C}$ .
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  $37^{\circ}\text{C}$ ).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed *Pseudomonas fluorescens* serum will be diluted (1/1,000-1/5,000) in PBS Tween containing 2.5g/l BSA,  $200\mu\text{l}$  by well plate (incubating during 2 hours at  $37^{\circ}\text{C}$ ).
5. Wash with PBS Tween (three times).
6.  $200\mu\text{l}$  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  $37^{\circ}\text{C}$ ).
7. Well plates will be rinsed with PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating  $200\mu\text{l}$  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  $50\mu\text{l}$  of 2M HCl.
9. The optical density will be measured at 492nm.

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

Membrane Blocking, Antibody Incubations and Detection of Proteins

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at  $37^{\circ}\text{C}$  while mixing.
2. Wash the membrane twice for 5 minutes in TBS Tween at  $37^{\circ}\text{C}$ .
3. Incubate the membrane with the antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at  $37^{\circ}\text{C}$ .
4. Wash the membrane three times for 5 minutes in TBS Tween at  $37^{\circ}\text{C}$ .
5. Incubate with a biotinylated secondary antibody diluted 1:1000 in TBS 0.5% Blocker for 2 hours at  $37^{\circ}\text{C}$ .
6. Wash the membrane three times for 5 minutes in TBS Tween at  $37^{\circ}\text{C}$ .
7. Incubate with Streptavidin-HRP  $1\mu\text{g/ml}$  in TBS 0.5% Blocker for 2 hours at room temperature.
8. Wash the membrane three times for 5 minutes in TBS at  $37^{\circ}\text{C}$ .
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) +  $50\mu\text{l}$  H<sub>2</sub>O<sub>2</sub> 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