



10451 ROSELLE STREET, #300, SAN DIEGO, CA 92121  
TELEPHONE (858) 642-1988 • FAX (858) 642-1989  
WWW.ATSBIO.COM • ATS@ATSBIO.COM

**Anti-Conjugated Phenyl Acetic Acid  
RAT POLYCLONAL  
AB-T099**

**ELISA protocol used to test conjugated Phenyl-acetic acid:**

1. Coating of conjugated Phenyl-acetic acid ( $10\mu\text{g/ml}$ ) 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-Tween (pH 7.3) containing 10% glycerol and 1g/l of BSA (Acros) (1h at  $37^{\circ}\text{C}$ ).
3. Wash with PBS-Tween (three times).
4. Preabsorbed conjugated Phenyl-acetic acid serum will be diluted (1/5,000-1/10,000) in PBS-Tween containing 1g/l BSA and 10% glycerol,  $200\mu\text{l}$  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-rat (Jackson) diluted (1/10,000) in a solution of PBS-Tween containing 5g/l 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.