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

Catalog Number: 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°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°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°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°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.