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**Anti-Gamma-Amino-Butyric Acid (GABA)
RAT POLYCLONAL**

Catalog Number: AB-T158

ELISA protocol used to test conjugated GABA:

1. Coating of conjugated GABA (10 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-Tween (pH 7.3) containing 10% glycerol and 1 g/l of BSA (Acros) (one hour at 37°C).
3. Wash with PBS-Tween (three times).
4. Preabsorbed conjugated GABA serum will be diluted (1/5,000-1/10,000) in PBS-Tween containing 1g/l BSA, 1g/l BSA-reduced glutaraldehyde and 10% glycerol, 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 1g/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 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.