ADVANCED TARGETING SYSTEMS

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**Anti-Conjugated Formiate**
**RAT POLYCLONAL**
**AB-T100**

**ELISA protocol used to test conjugated Formiate:**

1. Coating of conjugated Formiate (10$\mu$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 (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.5% of Tween (one hour at 37°C).
3. Wash with PBS (three times).
4. Preabsorbed conjugated Formiate serum will be diluted (1/5,000-1/10,000) in PBS Tween containing 2,5g/l BSA, 1g/l BSA-G and 10% of glycerol, 200$\mu$l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200$\mu$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$\mu$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$\mu$l of 2M HCl.
9. The optical density will be measured at 492nm.