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**Anti-Conjugated Adrenaline
RABBIT POLYCLONAL
AB-T069**

Example of ELISA protocol used to test conjugated adrenaline:

1. Coating of conjugated adrenaline ($15\mu\text{g/ml}$) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) (Acros) 0.001M, 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), 0.05% Tween 20 (Acros), and SMB 0.001M during one hour at 37°C .
3. Wash with PBS Tween (two times).
4. Anti-conjugated adrenaline antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, $200\mu\text{l}$ 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 sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with a 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, to obtain the different values.