



**Anti-Conjugated Succinate
RAT POLYCLONAL
AB-T101**

Example of ELISA protocol used to test conjugated succinic acid:

1. Coating of conjugated succinic acid ($15\mu\text{g/ml}$) 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 (pH 7.3) containing 2.5g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C .
3. Wash with PBS Tween (two times).
4. Preabsorbed succinic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of 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 (1/5,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol and 0.5% of Tween, 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 (IC 50).