ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Ascorbic Acid
RAT POLYCLONAL
AB-T151

Example of ELISA protocol used to test conjugated ascorbic acid:

1. Coating of conjugated ascorbic acid (15μg/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 1g/l of BSA (Acros) and 0.05% Tween 20 (Acros) during one hour at 37°C.
3. Wash with PBS Tween (three times).
4. Preabsorbed ascorbic acid antiserum will be diluted (1/2,000-1/5,000) in PBS tween containing 1g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 1g/l of BSA 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.
8. And finally the peroxidase will be developed by incubating 200μl 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 50μl of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values (IC 50).