ADVANCED TARGETING SYSTEMS

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Anti-Conjugated Nicotinamide
RAT POLYCLONAL
AB-T150

ELISA protocol used to test conjugated Nicotinamide:

1. Coating of conjugated Nicotinamide (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 phosphate buffer saline (PBS) (pH 7.3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3. Wash with PBS (three times).
4. Preabsorbed nicotinamide antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200μl by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS (three times).
6. 200μl of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA, will be applied by well plate (during one hour at 37°C) .
7. Well plates will be rinsed with a PBS solution containing 0.5% of 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.