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**Anti-Conjugated Nicotinamide  
RAT POLYCLONAL**

**Catalog Number:** AB-T150

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

1. Coating of conjugated Nicotinamide ( $15\mu\text{g/ml}$ ) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer  $0.05\text{M}$  (pH 9.6), during sixteen hours at  $4^\circ\text{C}$ .
2. Saturation of well plates with of a solution of phosphate buffer saline (PBS) (pH 7.3) containing  $2\text{g/l}$  of BSA (Acros) during one hour at  $37^\circ\text{C}$ .
3. Wash with PBS (three times).
4. Preabsorbed nicotinamide antiserum will be diluted ( $1/2,000$ - $1/5,000$ ) in PBS containing  $2\text{g/l}$  BSA and  $5\%$  of glycerol,  $200\mu\text{l}$  by well plate (incubating during 2 hours at  $37^\circ\text{C}$ ).
5. Wash with PBS (three times).
6.  $200\mu\text{l}$  of peroxidase-labeled goat anti-rat (Jackson) diluted ( $1/5,000$ ) in a solution of PBS containing  $5\text{g/l}$  of BSA, will be applied by well plate (during one hour at  $37^\circ\text{C}$ ).
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  $200\mu\text{l}$  by well plate of a citrate  $0.1\text{M}$ /phosphate  $0.2\text{M}$  (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  $2\text{M}$  HCl.
9. The optical density will be measured at  $492\text{nm}$ .