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**Anti-Conjugated Thioctic Acid
RAT POLYCLONAL**

Catalog Number: AB-T088

ELISA protocol used to test conjugated Thioctic acid:

1. Coating of conjugated Thioctic acid ($50\mu\text{g/ml}$) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C .
2. Wash with PBS (two times).
3. Preabsorbed Thioctic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA (lipid free), $200\mu\text{l}$ by well plate (incubating during 2 hours at 37°C).
4. Wash with PBS (three times).
5. $200\mu\text{l}$ of peroxidase-labeled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 2g/l of BSA (lipid free), will be applied by well plate (during one hour at 37°C).
6. Well plates will be rinsed with PBS containing 0.5% of Tween (three times).
7. 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.
8. The optical density will be measured at 492nm.