



11175-A FLINTKOTE AVENUE, SAN DIEGO, CA 92121
TELEPHONE (858) 642-1988 • FAX (858) 642-1989
WWW.ATSBIO.COM • ATS@ATSBIO.COM

Anti-Conjugated Ascorbic Acid RAT POLYCLONAL

Catalog Number: AB-T151

Example of ELISA protocol used to test conjugated ascorbic acid:

1. Coating of conjugated ascorbic 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 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, $200\mu\text{l}$ 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 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 $200\mu\text{l}$ 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).