



**Anti-Conjugated Homovanillic Acid  
RAT POLYCLONAL  
AB-T098**

**ELISA protocol used to test conjugated Homovanillic acid:**

1. Coating of conjugated Homovanillic acid ( $50\mu\text{g/ml}$ ) 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 PBS (pH 7.3) containing  $2\text{ g/l}$  of BSA (Acros) (one hour at  $37^\circ\text{C}$ ).
3. Wash with PBS (two times).
4. Preabsorbed conjugated Homovanillic acid serum will be diluted ( $1/5,000$ - $1/10,000$ ) in PBS containing  $2\text{g/l}$  BSA,  $5\%$  glycerol and  $0.2\text{g/l}$  of conjugated N.Acetyl-Cystéine (gemacbio),  $200\mu\text{l}$  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 ( $1/10,000$ ) in a solution of PBS -Tween containing  $5\text{g/l}$  BSA, will be applied by well plate (during one hour at  $37^\circ\text{C}$ ).
7. Well plates will be rinsed with PBS-Tween (three times).
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  $492\text{nm}$ .