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**Anti-Melatonin  
RABBIT POLYCLONAL**

**Catalog Number:** AB-T079

**Example of ELISA protocol used to test conjugated 5-Methoxytryptamine:**

1. Coating of conjugated 5-Methoxytryptamine ( $10\mu\text{g/ml}$ ) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at  $4^{\circ}\text{C}$ .
2. Saturation of well plates with a solution of PBS (pH 7.3) containing 1g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at  $37^{\circ}\text{C}$ ).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (three times).
4. Preabsorbed 5-Methoxytryptamine antiserum will be diluted (1/2,000-1/10,000) in PBS Tween containing 1g/l BSA, 1g/l of BSA-G and 10% of glycerol,  $200\mu\text{l}$  by well plate (incubating during 2 hours at  $37^{\circ}\text{C}$ ).
5. Wash with PBS Tween (three times).
6.  $200\mu\text{l}$  of peroxidase-labeled goat anti-rabbit (Jackson) diluted (1/10,000) in a solution of PBS Tween containing 1g/l of 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 0.1M/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.