



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

**Anti-Conjugated 5-Methoxytryptamine  
RABBIT POLYCLONAL**

**Catalog Number:** AB-T078

**Example of ELISA procol used to test conjugated 5-methoxytryptamine:**

1. Coating of conjugated 5-methoxytryptamine ( $15\mu\text{g/ml}$ ) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6) containing sodium metabisulfite (SMB) 0.001M, during sixteen hours at  $4^{\circ}\text{C}$ .
2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5g/l of BSA (Acros), 0.05% Tween 20 (Acros) and SMB 0.001M during one hour at  $37^{\circ}\text{C}$ .
3. Wash with PBS Tween (two times).
4. Anti-conjugated 5-methoxytryptamine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA, 10% of glycerol and SMB 0.001M, 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 sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol, 0.5% of Tween and SMB 0.001M, will be applied by well plate (during one hour at  $37^{\circ}\text{C}$ ).
7. Well plates will be rinsed with a 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, to obtain the different values.



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**Anti-Conjugated 5-Methoxytryptamine  
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**Example of Immunohistochemistry used to test conjugated 5-methoxytryptamine:**

*Detection of conjugated 5-methoxytryptamine in rat brain*

1. **Perfusion:** The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:  
solution A (30ml): 200-300ml/min  
solution B (500ml): 200-300ml/min  
Solution A: cacodylate 0.1M, sodium metabisulfite 10g/l, pH = 6.2  
Solution B: cacodylate 0.1M, sodium metabisulfite 10g/l and glutaraldehyde 3-5%, pH = 7.5
2. **Post fixation:** 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
3. **Tissue sectioning:** Cryostat or vibratome sections can be used.
4. **Reduction step:** Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
5. **Application of anti-conjugated 5-methoxytryptamine antibodies:** The final dilution is 1/1,000 to 1/5,000 in solution C containing triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.

*Note: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.*

6. **PAP procedure:**  
**Second antibody:** Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;  
**PAP:** Sections are incubated with 1/1000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;  
**Revelation:** Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated; 0.05% of H<sub>2</sub>O<sub>2</sub> is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.