

**Example of ELISA protocol used to test conjugated tyramine:**

1. Coating of conjugated tyramine (15 $\mu$ 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°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°C.
3. Wash with PBS Tween (two times).
4. Anti-conjugated tyramine 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 $\mu$ l by well plate (incubating during 2 hours at 37°C).
5. Wash with PBS Tween (three times).
6. 200 $\mu$ 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°C).
7. Well plates will be rinsed with a PBS Tween (three times).
8. And finally the peroxidase will be developed by incubating 200 $\mu$ 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 $\mu$ l of 2M HCl.
9. The optical density will be measured at 492nm, to obtain the different values .

**Example of Immunohistochemistry protocol used to test conjugated tyramine:**

1. Under deep anesthesia, each rat will be perfused through the ascending aorta with 100ml of 0.01M phosphate-buffered saline (PBS) followed by 200ml of fixative containing 2% glutaraldehyde, 1% sodium metabisulfite, and 0.25% picric acid in 0.1M PB.
2. The brain will be removed and cut into several blocks, which will be postfixed in the same fixative for 8h, followed by a rinse for 3 days in PBS containing 20% sucrose and 1% sodium metabisulfite.
3. Sections will be cut with a cryostat in traverse plane.
4. The sections will be incubated in polyclonal antiserum against p-tyramine diluted (1/1,000-1/5,000) in PBS containing 0.3% Triton X-100 and sodium azide at 4°C for 1 week; biotinylated rabbit IgG (Vector Laboratory, 1/1000) at 4°C for 12h; and avidin-biotin peroxidase complex (Vector Laboratory, 1/1000) at room temperature for 2h.
5. Peroxidase activity will be then revealed in 50mM Tris-HCl buffer (pH 7.6) containing 0.0003% H<sub>2</sub>O<sub>2</sub>, 0.01% 3,3'-diaminobenzidine-4HCl (DAB) and 1% nickel ammonium sulfate. The reaction will be terminated by washes in a Tris saline rinse.
6. Sections will be floated onto Tris solution on slides coated with 0.1% chromogelatin, dehydrated and coverslipped. Some serial sections will be stained by polyclonal anti-DA serum for the comparison of anti-p-tyramine staining.