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**Anti-Conjugated Riboflavin  
RAT POLYCLONAL**

**Catalog Number:** AB-T142

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

1. Coating of conjugated riboflavin ( $15\mu\text{g/ml}$ ) 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) during one hour at  $37^\circ\text{C}$ .
3. Wash with PBS (three times).
4. Preabsorbed riboflavin antiserum will be diluted ( $1/2,000$ - $1/5,000$ ) in PBS containing  $2\text{g/l}$  BSA and  $5\%$  of glycerol,  $200\mu\text{l}$  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 ( $1/5,000$ ) in a solution of PBS containing  $5\text{g/l}$  of BSA and  $0.5\%$  of Tween, will be applied by well plate (during one hour at  $37^\circ\text{C}$ ).
7. Well plates will be rinsed with a PBS solution containing  $0.5\%$  of Tween.
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  $492\text{nm}$ .

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**Example of Immunohistochemistry:**

*Perfusion protocol for Adult male monkeys (Macaca fascicularis) (weight 3-3.5 kg):*

1. The animals can be deeply anaesthetized with ketamine (8mg/kg, intramuscular) and sodium thiopental (500 mg/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 300 ml of cold physiologic saline (0.9% NaCl) and with the following fixative solutions:
  - a) 500 ml of 1% paraformaldehyde in 0.1 M phosphate-buffer (PB), pH 7.2, at room temperature (two minutes).
  - b) 2,500 ml of 4% paraformaldehyde in 0.1 M PB, pH 7.2, at 4°C (ten minutes).
  - c) 5,000 ml of cold 4% paraformaldehyde in 0.1 M PB, pH 7.2 (fifty minutes).
  - d) 2,000 ml of cold 5% sucrose in 0.1M PB, pH 7.2 (twenty minutes).
  - e) Dissect out the brains and place in 10% glycerol and 2% dimethylsulfoxide (DMSO) in 0.1M PB, pH 7.2, at 4°C for two days, and finally keep at the same temperature in 20% of glycerol and 2% DMSO in PB until the brains will be cut on a freezing microtome.

Around 50  $\mu$ m-thick serial sections will be obtained, kept at 4°C in PB (0.1 M, pH 7.2) containing 20% of glycerol and 30% of ethylene glycol, and processed for immunostaining.

*Example of Immunohistochemical Protocol:*

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH<sub>3</sub> (20%), H<sub>2</sub>O<sub>2</sub> (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H<sub>2</sub>O<sub>2</sub> and 66% of methanol).
2. Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)
3. Pre-incubate for 30 min in PBS containing 10% of normal horse serum and 0.3% of Triton X-100 (mixed solution).
4. Incubate at room temperature (1h 30min) and overnight at 4°C in the same mixed solution containing Riboflavin antiserum (diluted 1/500–1/1,000; as recommended dilutions).
5. Then, the sections will be wash in PBS (30 min).
6. After that we will incubate for 60 min at room temperature with biotinylated anti-rat immunoglobulin (Vector) diluted 1/200 in PBS.
7. Wash during 30 min with PBS.
8. Sections will be incubated for 1 h with a 1/100 diluted avidin-biotin-peroxidase complex (Vectastain).
9. After that we will wash the sections in PBS (30 min)
10. Wash with Tris-HCl buffer (pH 7.6)(10 min).
11. The tissue-bound peroxidase will be developed with H<sub>2</sub>O<sub>2</sub> using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).