



**Anti-Conjugated Ibuprofen  
RABBIT POLYCLONAL  
AB-T162**

**Example of ELISA protocol:**

1. Coating of Ibuprofen-BSA conjugate (10 $\mu$ g/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), during sixteen hours at 4°C.
2. Saturation of well plates with a solution of Phosphate Buffer Saline (PBS) (pH 7.3) containing 2.5g/l BSA (Acros) and 0.05% Tween 20 (across) (one hour at 37°C).
3. Wash with PBS containing 0.05% of Tween (PBS Tween) (three times).
4. Anti-conjugated Ibuprofen antibodies will be diluted (1/5,000-1/10,000) in PBS Tween containing 2.5g/l BSA, 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 goat anti-rabbit (Biorad) diluted (1/10,000) in a solution of PBS Tween containing 2.5g/l BSA, will be applied by well plate (during one hour at 37°C).
7. Well plates will be rinsed with 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.

**Example of Western blot protocol (Membrane blocking, antibodies incubations and detection):**

1. Saturate the blot membrane with TBS + 5% Blocker for 1 hour at 37°C while mixing
2. Wash the membrane twice for 5 minutes in TBS Tween at 37°C
3. Incubate the membrane with anti-conjugated Ibuprofen antibodies diluted 1/1,000 – 1/2,000 in TBS 0.5% Blocker for 2 hours at 37°C
4. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
5. Incubate with a biotinylated secondary antibody diluted (1/1,000-1/2,000) in TBS 0.5% Blocker for 2 hours at 37°C
6. Wash the membrane three times for 5 minutes in TBS Tween at 37°C
7. Incubate with Streptavidin-HRP 1 $\mu$ g/ml in TBS 0.5% Blocker for 2 hours at room temperature
8. Wash the membrane three times for 5 minutes in TBS at 37°C
9. Incubate in TBS (200ml) + (50mg DAB in 25ml methanol) + (150mg 4-chloro-1-naphtol in 25ml methanol) + 50 $\mu$ l H<sub>2</sub>O<sub>2</sub> 30% for a maximum of 30 minutes in the dark
10. Stop the reaction by addition of distilled water

Blocker = skim milk (Biorad 170-6404)

TBS = 20mM Tris base, 0.5M NaCl, pH 7.5

TBS Tween = TBS + 0.05% Tween 20

**Anti-Conjugated Ibuprofen  
RABBIT POLYCLONAL  
AB-T162**

**Example of immunohistochemistry protocol:**

*Example of Perfusion protocol for Adult male Sprague Dawley (weight around 0.5 kg):*

1. The animals can be deeply anaesthetized (for example with urethane-0.5-1.5g/kg, intraperitoneal).
2. Heparinized, and perfused via the ascending aorta with 50 ml of MES (2-Morpholinoethanesulfonic acid monohydrate; Fluka)  $10^{-1}$  M, pH 5.4, and with the following solutions:
  - a) 200 ml of a solution containing MES  $10^{-1}$  M, pH 5.4 and ECD [1-(3-Dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride; Acros]  $10^{-1}$  M (two minutes).
  - b) 800-1000 ml of phosphate buffer (PB) pH 7.2 (eight minutes)
  - c) 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
  - d) Dissect out the organs and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4°C for twelve to sixteen hours.

*Example of immunohistochemical protocol:*

1. In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing  $\text{NH}_3$  (20%),  $\text{H}_2\text{O}_2$  (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of  $\text{H}_2\text{O}_2$  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 anti-conjugated Ibuprofen antibodies (diluted 1/1,000 to 1/5,000; as recommended dilution).
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-rabbit 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  $\text{H}_2\text{O}_2$  using 3, 3' diaminobenzidine as chromogen.
12. Finally the sections will be rinsed with PBS and coverslipped with PBS/Glycerol (1/1).