



## ANTI-CONJUGATED GLYOXAL ANTIBODIES

**CATALOG NUMBER : AP167**

**TARGET :** Conjugated Glyoxal

**IMMUNOGEN :** Synthetic Glyoxal conjugated to bovine serum albumin (BSA)

**SPECIFICITY :** Using a conjugate Glyoxal-BSA, antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

<i>Compounds</i>	<i>Cross-reactivity ratio (a)</i>
Glyoxal-BSA	1
MethylGlyoxal	1/>50,000
Glutaraldehyde-BSA	1/>50,000
Malondialdehyde-BSA	1/>50,000
Acrolein-BSA	1/>50,000

(a) : Glyoxal-BSA concentration /conjugated close related structures concentration at half displacement.  
BSA = Bovine Serum Albumin.

**RAISED IN :** Rabbit

**CLONALITY :** Polyclonal

**ISOTYPE :** IgG

**PURITY :** Antiserum previously preabsorbed on protein carriers, and purified

**FORM :** Lyophilized

**APPLICATIONS :** Optimal dilutions should be determined by each laboratory for each application.

**RESEARCH AREAS :** Neuroscience, biochemistry (metabolic ways)

**STORAGE INSTRUCTIONS :** Lyophilized vial must be stored at 4°C in a dry area. After reconstitution with 50µl of distilled water and 50µl of glycerol, the aliquot can be stored at -20°C, and is stable at least 2 years.

**CORRESPONDING ANTIGEN :** Gemacbio sells the corresponding antigen: Glyoxal conjugate (code number: AG167)

## **EXAMPLE OF MATERIAL AND METHODS**

### **• Example of immunohistochemistry protocol**

**Perfusion** (Example 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-Perfused via the ascending aorta with 50 ml of NaCl 9g/l (Heparinized) and pass through the system 800-1000 ml of cold 4% paraformaldehyde (Merck) in 0.1 M PB, pH 7.2-7.4, (ten minutes).
- 3-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.

#### **Immunohistochemistry**

- 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 2-10% (variable to adjust) 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 the diluted anti-conjugated Glyoxal antibodies(1/1,000-1/5,000).
- 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-(species) 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) in the mixed solution.
- 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).

### **• Immunoblot protocol :**

#### **Membrane Blocking, Antibody Incubations and Detection of Proteins**

- 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 Glyoxal 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 1:1000 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 1µg/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) + 50µl 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