ANTI-CONJUGATED GLYOXAL ANTIBODIES

CATALOG NUMBER : AP167

LOT NUMBER : 09083101

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 :

Compounds	*Cross-reactivity ratio (a)*
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 preabsobed 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)

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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 trhough 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 4ºC 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_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_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 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 4º 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) immunogammaglobulin (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_2O_2 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, Antiboby 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_2O_2 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

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