

Anti-Conjugated Glyoxal Antibodies

Product Information Sheet
AP167GE



SUMMARY

shipped at -20 °C; store at -20 °C

For research use only

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 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).

Corresponding Antigen: Mobitec sells the corresponding antigen: Glyoxal conjugate (code number: AG167GE)

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 - 1.5 g/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 NH₃ (20%), H₂O₂ (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H₂O₂ 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 H₂O₂ 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 1 µg/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 (200 ml) + (50 mg DAB in 25 ml methanol) + (150 mg 4-chloro-1-naphthol in 25 ml methanol) + 50 µl H₂O₂ 30% for a maximum of 30 minutes in the dark.
- 10 Stop the reaction by addition of distilled water.

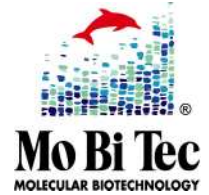
Blocker = skim milk (Biorad 170-6404)

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

TBS Tween = TBS + 0.05% Tween 20

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Order Information, Shipping and Storage

Order#	Product	Quantity
AP167GE	Anti-Conjugated Glyoxal Antibodies	50 µl
shipped at -20 °C; store at -20 °C		

Contact and Support

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