POLYCLONAL ANTI-CONJUGATED FOLIC ACID ANTIBODIES (rat)

Data Sheet

*Code number : **AP099***

TARGET : Folic Acid (Vitamin B9)

IMMUNOGEN : Synthetic Folic Acid conjugated to bovine serum albumin (BSA)

SPECIFICITY : Using a conjugate Folic acid-protein carrier (BSA), antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

Compound	*Cross-reactivity ratio* [(a)]
Folic acid-BSA	1
Folinic acid-BSA	1/>900
Tetrahydrofolic acid-BSA	1/>2,000
Methotrexate-BSA	1/>50,000
Riboflavin-BSA	1/>50,000
BSA	1/>50,000

(a) : Folic acid-BSA concentration/unconjugated or conjugated compound concentration at half displacement.

RAISED IN : Rat

CLONALITY : Polyclonal

ISOTYPE : Ig G

PURITY : Antiserum previously preabsobed on protein carriers, and purified.

FORM : Lyophilized

TESTED APPLICATIONS : ELISA, IMMUNOHISTOCHEMISTRY

APPLICATIONS NOTES : Recommended dilutions for ELISA (1/2,000-1/5,000), for Immunohistochemistry (1/500-1/1,000).

RESEARCH AREAS : Neuroscience, neurodegenerative disorders, cardiovascular diseases, biochemistry (metabolic ways).

NOTES : Principal name Folic acid or pteroylglutamic acid

STORAGE INSTRUCTIONS : After reconstitution with 50µl of distilled water and 50 µl of glycerol, the aliquot can be repeated freezed (up to five times), and stable at least 2 years.

Corresponding antigen

Gemac sells the corresponding antigen:

Folic acid conjugate (code number: AG099)

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EXAMPLES OF MATERIAL AND METHODS

Example of ELISA procol used to test conjugated Folic acid :

1-Coating of conjugated folic acid (15µg/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 of a solution of PBS (pH 7,3) containing 2g/l of BSA (Acros) during one hour at 37°C.
3-Wash with PBS (three times).
4-Preabsorbed folic acid antiserum will be diluted (1/2,000-1/5,000) in PBS containing 2g/l BSA and 5% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5-Wash with PBS (three times).
6-200µl of peroxidase-labelled goat anti-rat (Jackson) diluted (1/5,000) in a solution of PBS containing 5g/l of BSA and 0.5% of Tween, will be applied by well plate (during one hour at 37°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 200µl 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 50µl of 2M HCl.
9-The optical density will be mesured at 492nm.

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 4ºC (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).
d) Dissect out the brains and place in 10% glycerol and 2% dimethylsufoxide (DMSO) in 0.1M PB, pH 7.2, at 4ºC 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 µm-thick serial sections will be obtained, kept at 4º 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

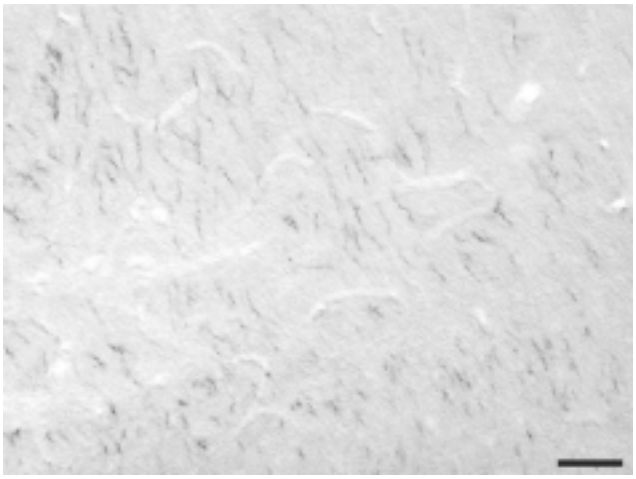
1-In order to avoid possible interference with endogenous peroxidase, free-floating sections will be treated with distilled water containing NH_3 (20%), H_2O_2 (30%) and NaOH (1%) for 20 min (other method is using a solution with 33% of H_2O_2 and 66% of methanol).
2-Then, wash the sections for 20 min in 0.15 M phosphate-buffered saline (PBS) (pH 7.2)

3-Pre-incubate for 30 min in PBS containing 10% 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 folic acid 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 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).

References

- MANGAS A., COVEÑAS R., GEFFARD K., GEFFARD M., MARCOS P., INSAUSTI R. and DABADIE M.P. Folic acid in the monkey brain: an immunocytochemical study. Neurosc. Let. (2004); 362 : 258-261.

Here, we show an example of the immunoreactive fibers that have been found using this protocol.



Folic Acid-immunoreactive fibers located in the ventro-lateral part of the pulvinar nucleus of the monkey brain. Scale bar: 50µm.

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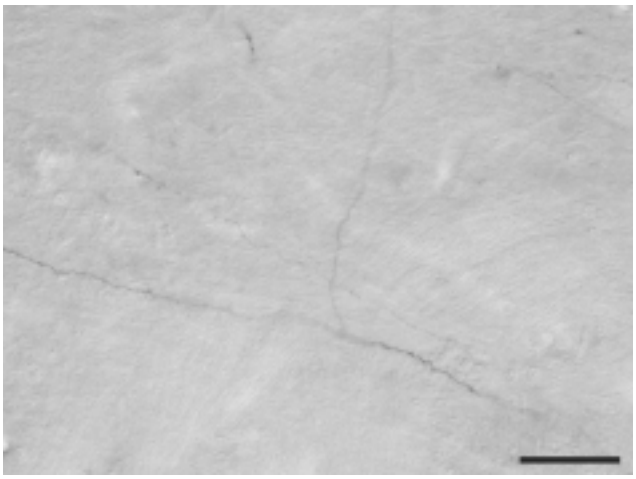

Immunoreactive fibers containing Folic acid in the dorsal mesencephalic region of the monkey brain.

Fibers located between the ventricul and the lateral geniculate nucleus of the thalamus. Scale bar: 50µm

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