コスモ・バイオ株式会社

ANTI-CONJUGATED L-ARGININE ANTIBODIES

CATALOG NUMBER : AP022

TARGET : Conjugated L-arginine

IMMUNOGEN : Synthetic L-arginine conjugated to protein carrier (Pc)

SPECIFICITY : Using a conjugate L-arginine-(Pc), antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

Compound	*Cross-reactivity ratio (a)*
Arginine-G-(Pc)	1
Glutamine-G-(Pc)	1/> 100,000
Asparagine-G-(Pc)	1/> 100,000
Lysine-G-(Pc)	1/> 100,000

(a) : Arginine-G-(Pc) concentration / other conjugated aminoacids concentration at half displacement ; G = Glutaraldehyde

RAISED IN : Rabbit

CLONALITY : Polyclonal

ISOTYPE : IgG

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

FORM : Lyophilized

TESTED APPLICATIONS : Elisa, Immunocytochemistry

APPLICATIONS NOTES :
Recommended dilutions for Immunocytochemistry (1/1,000-1/5,000)
Recommended dilutions for Elisa (1/1,000-1/5,000)
Recommended dilutions for Western Blot (1/1,000-1/2,000)

RESEARCH AREAS : Biochemistry, Radical processes, Degenerative diseases, Neurobiology

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

CORRESPONDING ANTIGEN :
Gemacbio sells the corresponding antigen : L-Arginine conjugate (code number : AG022)

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REFERENCES :
• KING M.G., GEFFARD M., CHAUVEAU J., GAFFORI O., LE MOAL M. and MUYARD J.P. Des tyr-g-endorphin (DTgE) and pineal levels of melatonin and arginine vasopressin (AVP). Eur. J. Pharmacol., (1981) 76, 271-274.
• KING M.G., LE MOAL M., STINUS L. and GEFFARD M. Central administration of arginine-vasotocin: effect of exploratory behavior in the rat. Psychopharmacology, (1982) 76, 40-43.

EXAMPLE OF MATERIAL AND METHODS

• Example of ELISA protocol used to test conjugated L-arginine

1- Coating of conjugated L-arginine (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 2.5g/l of BSA (Acros) and 0.05%Tween 20 (Acros) during one hour at 37°C.
3- Wash with PBS Tween (two times).
4- Anti-conjugated L-arginine antibodies will be diluted (1/1,000-1/5,000) in PBS containing 2.5g/l BSA and 10% of glycerol, 200µl by well plate (incubating during 2 hours at 37°C).
5- Wash with PBS Tween (three times).
6- 200µl of peroxidase-labelled sheep anti-rabbit (Bio-Rad) diluted (1/10,000) in a solution of PBS containing 2.5g/l BSA, 10% of glycerol 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 Tween (three times).
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, to obtain the different values.

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• Example of cytochemistry application used to test conjugated L-arginine :

PERFUSION PROTOCOL FOR ADULT MALE SPRAGUE DAWLEY (WEIGHT AROUND 0.5KG) :

1- The animals can be deeply anesthetized with for example urethane (0.5-1.5g/kg, intraperitoneal).
2- Heparinized, and perfused via the ascending aorta with 100ml of cold physiologic saline (0.9% NaCl) and with the following fixative solution :
a) 300ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (two minutes).
b) 600ml of cold 4% paraformaldehyde and 2% glutaraldehyde in 0.1M phosphate-buffer (PB), pH 7.2 (ten minutes).
c) Dissect out the brains and place in a solution of 4% paraformaldehyde in 0.1M PB, pH 7.2, at 4ºC for twelve to sixteen hours.
d) Before the brains will be cut on a freezing microtome, we must include the brain in growing concentrations of sucrose (a first bain of 5% of sucrose in PBS until the brains sank), after that we will repeat the same process in a solution with a higher level of sucrose (10%), 20%, 25% and finally 30%.

Around 50µm-thick serial sections will be obtained, kept at 4º C in PBS (0.1M, pH 7.2) 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 0.15M 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 (1h30min) and overnight at 4ºC in the same mixed solution containing L-arginine antiserum (diluted 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-rat immunogammaglobulin (Vector) diluted 1/200 in PBS.
7- Wash during 30 min with PBS.
8- Sections will be incubated for 1h 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 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).

Gemacbio sells the citrulline and agmatine antibodies raised in rabbit : used together, these tools could be helpful for immunocytochemistry double labelling.

• Western blot : Antibodies can be used for western blot

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