**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-Glutamic Acid Decarboxylase (GAD) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M018-3	9A6	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Glutamic acid decarboxylase (GAD) catalyzes formation of γ-Aminobutyric acid (GABA), which is the inhibitory neurotransmitter, and is present in brain as well as several tissues outside the central nervous system. Biological functions of GAD and GABA extend beyond regulation of neurotransmission to include effects on the immune system as well as modulation of cell proliferation, protein synthesis, and metabolism. Two isoforms of GAD, GAD65 and GAD67 each derives from a single separate gene, were isolated from human fetal brain λgt11 cDNA library.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human GAD65 protein corresponding to full length amino acids (1-585 aa).

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with GAD65 and GAD67 on Western blotting and Immunohistochemistry. Because this antibody detects 65 kDa and 67 kDa of mouse and rat GAD by Western blotting on total cell lysate from brain, it will also react with human GAD.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; 1-10 µg/mL
Immunocytochemistry; Not tested
Flow Cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

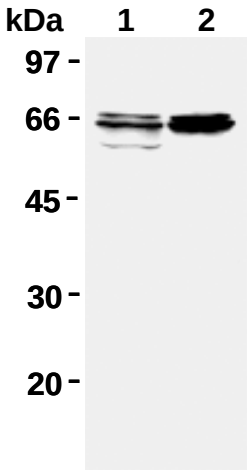
SPECIES CROSS REACTIVITY:

Species	Human*	Mouse	Rat
Tissues	Not tested	Brain	Brain
Reactivity on WB		+	+

*Reactivity of this antibody (clone 9A6) to human is not confirmed in our laboratory. However, it is reported that this antibody reacts with human brain in Immunohistochemistry [1].

REFERENCES:

1) Becker, A. B., *et al*., *J. Neuropahtol. Exp. Neurol.* **76**, 665-675 (2017) [IHC]
2) Volgin, D. V., *et al*., *PLoS One* **9**, e86545 (2014) [IHC]
3) Kuwajima, T. *et al*., *J. Neurosci*. **26**, 5383-5392 (2006) [WB, IHC]
4) Kasuga, A. *et al*., *J. Autoimmunity* **9**, 105-111 (1996)
5) Daw, K. *et al*., *J. Immunol*. **156**, 818-825 (1996)
6) Grubin, C. E. *et al*., *Diabetologia* **37**, 344-350 (1994)
7) Mauch, L. *et al*., *J. Biochem*. **113**, 699-704 (1993)
8) Deaizpurua, H. J., *et al*., *Diabetes* **41**, 1182-1187 (1992)
9) Bu, D. F., *et al*., *PNAS*. **89**, 2115-2119 (1992)
10) Michelsen, B. K., *et al*., *PNAS*. **88**, 8754-8758 (1991)
11) Karlsen, A. E., *et al*., *PNAS*. **88**, 8337-8341 (1991)



Western blot analysis of GAD expression in mouse (1) and rat (2) brain using M018-3.

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

PROTOCOLS

SDS-PAGE & Western Blotting

1) Boil the samples for 2 minutes and centrifuge. Load 20 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
3) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
4) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
9) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Mouse or rat brain)

Immunohistochemical staining for paraffin-embedded sections : SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific antibody staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8.
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8.
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.