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

MONOCLONAL ANTIBODY

Anti-Neuropsin (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M021-3	MabB5	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Neuropsin is a trypsin-type, extracellular, serine protease that was first cloned from mouse limbic brain as a factor related to neural plasticity. Physiological studies indicated that kindling stimulation induced neuropsin mRNA and its protease activity also increased in limbic areas during kindling and long-term potentiation. Subsequent study indicated that neuropsin mRNA localized not only in the limbic brain, but also in developing organs and adult keratinocytes. Recent immunohistochemical analysis demonstrated that neuropsin localized exclusively in the keratinizing epithelia of the epidermis and various stratified mucous membranes. During development, neuropsin was found in the various epithelia at embryonic days 14.5-15.5, prior to formation of the stratum corneum. Much stronger expression of neuropsin is observed in nude mouse skin and mucous membranes than in normal mice. Those studies suggested that neuropsin is related to differentiation of keratinocytes and the process of keratogenesis.

SOURCE: This antibody was purified from rat ascites fluid using protein A agarose. This hybridoma (clone MabB5) was established by fusion of rat myeloma cell Y3Ag1.2.3 with Wister rat splenocyte immunized with the recombinant protein.

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 mouse neuropsin (32 kDa) on Western blotting with total cell lysate from neuropsin overexpressed insect cell.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Samples	Not tested	Recombinant, transfectant	Not tested
Reactivity on WB		+	

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APPLICATIONS-CONFIRMED:
Western blotting; 1-5 µg/mL for a chemiluminescence detection system
Immunoprecipitation; 2 µg/sample

APPLICATIONS-REPORTED:
Immunohistochemistry (Frozen section); Reference 8)
Immunocytochemistry; Reference 4)
Neutralizing activity; Reference 2) and 3)
Functional activity (inhibition); Reference 1)

Detailed procedures are provided in the following **PROTOCOL**.

REFERENCES:
1) Herring, A., *et al., Alzheimers Dement.* **12**, 1273-1287 (2016) [WB, Function]
2) Tamura, H., *et al., J. Physiol*. **570**, 541-551 (2006) [NT]
3) Matsumoto-Miyai, K., *et al., J. Neurosci.* **23**, 7727-7736 (2003) [NT]
4) Oka, T., *et al., J. Biol. Chem.* **277**, 14724-14730 (2002) [IC]
5) Kato, K., *et al., J. Biol. Chem.* **276**, 14562-14571 (2001) [IP]
6) Momota, Y., *et al., Eur. J. Neurosci*. **10**, 760-764 (1998)
7) Shimizu, C., *et al., J. Biol. Chem.* **273**, 11189-11196 (1998) [WB, IP]
8) Inoue, N., *et al., J. Invest. Dermatol.* **110**, 923-931 (1998) [IHC]
9) Chen, Z. L., *et al., J. Histochem. Cytochem.* **46**, 313-320 (1998)
10) Okabe, A., *et al., Brain Res*. **728**, 116-120 (1996)
11) Chen, Z. L., *et al., J. Neurosci.* **15**, 5088-5097 (1995)

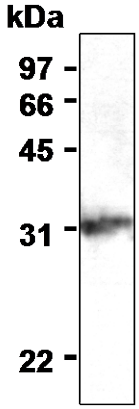
Clone MabB5, also known as mAbB5 or B5mAb, is used in reference number 1) - 8).

PROTOCOL:
SDS-PAGE & Western Blotting
1) Wash cells at a concentration of 1 x 10^7 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.
3) 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 manufacturer's manual for precise transfer procedure.
4) 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.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2)

containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)

6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with HRP-conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 10 seconds. Develop the film under usual settings. The conditions for exposure and development may vary.

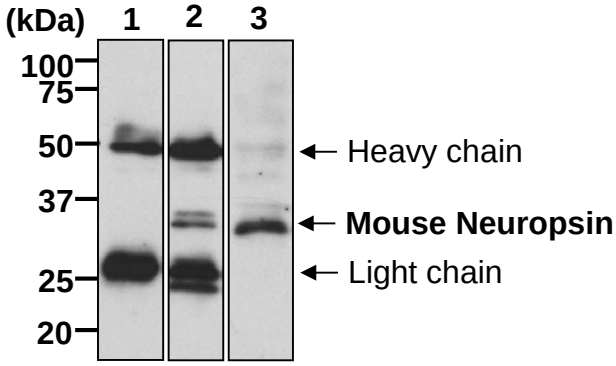
(Positive control for Western blotting; Recombinant)



Western blot analysis of mouse Neuropsin expression in recombinant protein using M021-3.

Immunoprecipitation

1) Wash 1x10^7 cells 3 times with PBS and suspend with 10 volumes of Extraction buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors.
2) Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
3) Centrifuge the tube at 10,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
4) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 1 hour at room temperature.
5) Add 20 µL of 50% protein G agarose beads resuspended in the Extraction buffer. Mix well and incubate with gentle agitation for 1 hour at room temperature.
6) Wash the beads 5 times with the Extraction buffer (centrifuge the tube at 2,500 x g for 10 seconds).
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.
8) (See **SDS-PAGE & Western blotting**.)



Immunoprecipitation of mouse Neuropsin from 293T transfectant with rat IgG2a (1) or M021-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M021-3. Lane 3 is the input sample.*

RELATED PRODUCTS:

M020-3	Anti-Neuropsin (Mouse) mAb (MabF12)
M021-3	Anti-Neuropsin (Mouse) mAb (MabB5)
M081-3	Rat IgG2a (isotype control) (2H3)
M081-4	Rat IgG2a (isotype control)-FITC (2H3)
M081-5	Rat IgG2a (isotype control)-PE (2H3)
M081-8	Rat IgG2a (isotype control)-Agarose (2H3)