

MONOCLONAL ANTIBODY

Anti-SynCAM (TSLC1/CADM1) mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|----------|-------|-------------|----------|---------------|
| CM004-3 | 3E1 | Chicken IgY | 100 µL | 1 mg/mL |

BACKGROUND: SynCAM (Synaptic Cell Adhesion Molecule), also known as TSLC1 (tumor suppressor in lung cancer-1), SgIGSF, or CADM1, is a homophilic, transmembrane Ig-domain containing protein with intracellular PDZ protein-binding motifs. Although originally identified as a tumor suppressor of small lung cell carcinomas, SynCAM appears to be primarily involved in intracellular adhesion and synapse formation. The majority of SynCAM is localized to synaptic sites where it initiates synaptic assembly and synapse differentiation throughout the central nervous system. SynCAM also mediates the cellular adhesion of spermatogenic cells to Sertoli cells and mast cells to fibroblasts.

SOURCE: This antibody was purified from hybridoma (clone 3E1) supernatant using anti-IgY affinity column. This hybridoma was established by fusion of chicken B cell line MUH1 cell with chicken splenocyte immunized with recombinant SynCAM-Fc fusion 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 extracellular domain of SynCAM on Flow cytometry, Western blotting, Immunoprecipitation and Immunohistochemistry.

APPLICATIONS:

Western blotting: 1 µg/mL for chemiluminescence detection system

Immunoprecipitation: 10 µg/200 µL of cell extract from 5 x 10⁶ cells

Immunohistochemistry: 10 µg/mL

Heat treatment is necessary for paraffin embedded sections.

Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)

Immunocytochemistry: Not tested*

*It is reported that clone 3E1 can be used in this application in the reference number 3), 4) and 6).

Flow cytometry: 1 µg/mL (final concentration)

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 |
|------------------|--------------|-----------------------|------------|
| Cell and Tissue | Transfectant | GC-1 spg cell, brain* | Not tested |
| Reactivity on WB | + | + | |

*Reactivity of clone 3E1 to mouse is not confirmed in our laboratory. However, it is reported that this clone reacts with GC-1 spg cells (mouse germ cell line)¹⁾ and mouse brain²⁾.

REFERENCES:

- 1) Gao, Y. and Lui, W. Y., *PLoS One* **8**, e64316 (2013) [WB]
- 2) Hagiya, M., *et al.*, *Am. J. Pathol.* **174**, 2278-2289 (2009) [WB, IP]
- 3) Hasstedt, S. J., *et al.*, *Blood* **114**, 3084-3091 (2009) [IC, IHC]
- 4) Hollins, F., *et al.*, *J. Immunol.* **181**, 2772-2780 (2008) [IP, IC]
- 5) Fujita, E., *et al.*, *Am. J. Pathol.* **171**, 1800-1810 (2007) [IH]
- 6) Wakayama, T., *et al.*, *Biol. Reprod.* **76**, 1081-1090 (2007) [IC]
- 7) Fogel, A. I., *et al.*, *J. Neurosci.* **27**, 12516-12530 (2007) [WB, IP]
- 8) Yang, W., *et al.*, *J. Immunol.* **176**, 1238-1243 (2006)
- 9) Furuno, T., *et al.*, *J. Immunol.* **174**, 6934-6942 (2005)
- 10) Sara, Y., *et al.*, *J. Neuroscience* **25**, 260-270 (2005)
- 11) Biederer, T., *et al.*, *Science* **297**, 1525-1531 (2002)

Clone 3E1 is used in the reference number 1)-8).

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

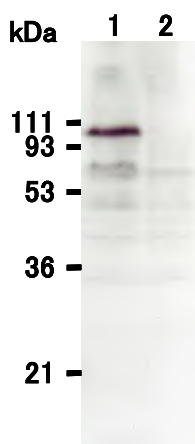
PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.

- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with the 1:5,000 HRP-conjugated anti-chicken IgY (CHEMICON; code no. AP162P) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (5 minutes x 6 times).
- 11) 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.
- 12) Expose to an X-ray film in a dark room for 10 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blot analysis of SynCAM expression in SynCAM transfected L cells (1) and L cells (2) using CM004-3.

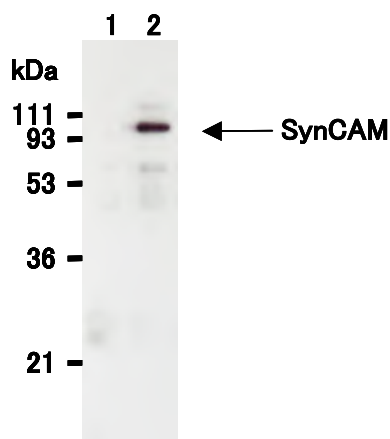
Immunoprecipitation

- 1) Wash the Biotin labeled transfectant cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggested in the

APPLICATIONS into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 μL of 50% anti-IgY agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.

- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Resuspend the agarose with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 4)-6) 2-4 times.
- 8) Resuspend the beads in 20 μL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 11) Incubate the membrane with HRP-conjugated streptavidin diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
- 13) 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.
- 14) Expose to an X-ray film in a dark room for 10 seconds. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Transfectant)

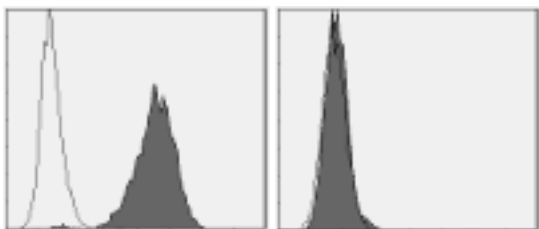


Immunoprecipitation of SynCAM from L cells (1) and SynCAM transfected L cells (2) with CM004-3. After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 2) Resuspend the cells with washing buffer (5x10⁶ cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 4) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 30 µL of 1:100 FITC conjugated anti-chicken IgY (CHEMICON; code no. AP162F) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of SynCAM expression on L cells (right) and SynCAM transfected L cells (left). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of CM004-3 to the cells.

(Positive control for Flow cytometry; Transfectant)

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) Heat treatment

Heat treatment by microwave oven:

Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

- 5) Remove the slides from the citrate buffer and cover each section with 3% H₂O₂ for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
- 6) Remove the slides from PBS, wipe gently around each section and cover tissues with the blocking reagent to block non-specific antibody staining. Do not wash.
- 7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
- 8) Incubate the sections for 1 hour at room temperature.
- 9) Wash the slides 3 times in PBS for 5 minutes each.
- 10) Wipe gently around each section and cover tissues with 1:100 Biotin conjugated anti-chicken IgY (CHEMICON; code no. AP162B). Incubate for 10 minutes at room temperature. Wash as in step 9).
- 11) Wipe gently around each section and cover tissues with the streptavidin-peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 9).
- 12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H₂O₂ in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 13) Wash the slides in water for 5 minutes.
- 14) 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.
- 15) Now ready for mounting.

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