

MONOCLONAL ANTIBODY

Anti-Desmoglein 3 (Mouse) mAb (No Azide)

| Code No. | Clone | Subclass | Quantity | Concentration |
|----------|-------|------------|----------|---------------|
| D217-3 | AK9 | Mouse IgG1 | 100 µL | 1 mg/mL |

BACKGROUND: Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease of the skin and mucous membranes. In patients of PV, IgG autoantibodies against the cell surface of keratinocytes in stratified squamous epithelia play a major pathogenic role in loss of cell-cell adhesion. The autoimmune target of PV is desmoglein 3 (Dsg3), which is a member of the cadherin family of calcium-dependending, transmembrane glycoprotein.

SOURCE: This antibody was purified from hybridoma (clone AK9) supernatant using protein A agarose. This hybridoma was established by fusion of splenocytes from PV model mouse and P3 mouse myeloma cells.

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

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with mouse Dsg3.

APPLICATIONS:

- Western blotting; Not tested
- Immunoprecipitation; Clone AK9 is used in reference 2).
- Immunohistochemistry; 0.01 µg/mL
- Immunocytochemistry; 0.01 µg/mL
- 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 | Skin | Oral mucosa | Not tested |
| Reactivity on IHC | - | + | |

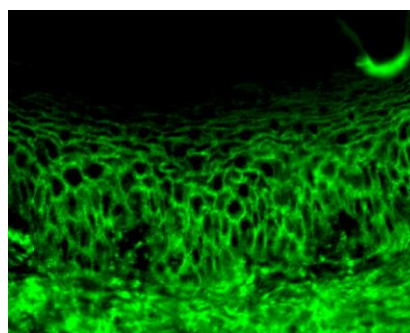
RELATED PRODUCTS:

- D218-3 Anti-Desmoglein 3 (Mouse) mAb (No Azide) (AK18)
- D219-3 Anti-Desmoglein 3 (Mouse) mAb (No Azide) (AK23)
- M075-3 Mouse IgG1 (isotype control) (2E12)

REFERENCES:

- 1) Takahashi, H., *et al.*, *J. Immunol.* **182**, 1740-1745 (2009)
- 2) Tsunoda, K., *et al.*, *J. Immunol.* **170**, 2170-2178 (2003)
- 3) Yamaguchi, T., *et al.*, *Infect. Immun.* **70**, 5835-5845 (2002)

Clone AK9 is used in these references.



Immunohistochemical detection of mouse Dsg3 on frozen section of mouse oral mucosa with D217-3.

This data was kindly provided by Dr. Amagai, M.D. Ph.D. and Dr. Tsunoda Ph.D. (The Department of Dermatology, School of Medicine, Keio University, Tokyo)

PROTOCOLS:

Immunohistochemical staining for frozen sections

- 1) Cover tissues with the D217-3 diluted with dilution buffer (TBS containing 0.5 mM CaCl₂, 1% BSA) as suggested in the **APPLICATIONS**.
- 2) Incubate the sections for 1 hour at room temperature.
- 3) Wash the slides with washing buffer (TBS containing 0.5 mM CaCl₂) (5 minutes x 3 times).
- 4) Wipe gently around each section and cover tissues with FITC conjugated anti-mouse IgG diluted by dilution buffer.
- 5) Incubate the sections for 1 hour at room temperature.
- 6) Wash the slides with washing buffer (5 minutes x 3 times).
- 7) Now ready for mounting.

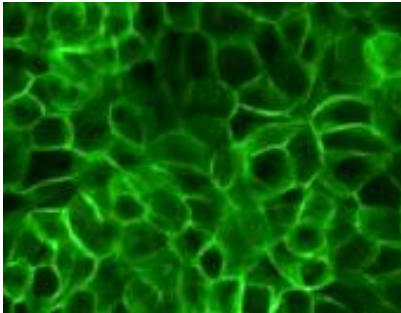
(Positive control for Immunohistochemistry; mouse oral mucosa)

Immunocytochemistry for living keratinocytes

- 1) Prepare 80-90% confluent mouse keratinocyte cells in chamber slide.

- 2) Wash the cells 2 times with DMEM (FCS free).
- 3) Add primary antibody diluted with medium as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes on ice (Optimization of antibody concentration or incubation condition is recommended if necessary).
- 4) Aspirate medium by aspirator.
- 5) Wash the cells with PBS (5 minutes x 3 times).
- 6) Apply Methanol into each well.
- 7) Incubate for 20 minutes at -30°C.
- 8) Aspirate Methanol by aspirator.
- 9) Wash the cells with PBS (5 minutes x 3 times).
- 10) Add FITC conjugated anti-mouse IgG antibody diluted with PBS for 30-60 minutes at room temperature. Keep out light by aluminum foil.
- 11) Wash the cells with PBS (5 minutes x 3 times).
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for immunocytochemistry; PAM212)



Immunocytochemical detection of Dsg3 on living mouse keratinocyte cell line (PAM212 cells) with D217-3.

This data was kindly provided by Dr. Amagai, M.D. Ph.D. and Dr. Tsunoda Ph.D. (The Department of Dermatology, School of Medicine, Keio University, Tokyo)