

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity	Concentration
D219-3	AK23	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 AK23) supernatant using protein A. 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 and human Dsg3, and has the pathogenic activities in mice*.
*This inducibility is reported in reference 5).

APPLICATIONS:

Western blotting; Not recommended

Immunoprecipitation; Clone AK23 is used in reference 5)

Immunohistochemistry; 1 µg/mL*

Immunocytochemistry; 1 µg/mL*

*The optimal antibody concentration should be determined depending on experimental conditions.

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	+	+	

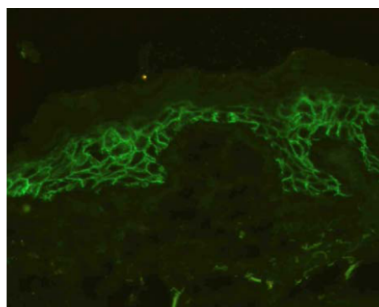
REFERENCES:

- 1) Heupel, W. M., *et al.*, *J. Biol. Chem.* **284**, 8589-8595 (2009)
- 2) Chernyavsky, A. I., *et al.*, *J. Biol. Chem.* **282**, 13804-13812 (2007)
- 3) Calkins, C. C., *et al.*, *J. Biol. Chem.* **281**, 7623-7634 (2006)
- 4) Kottke, M. D., *et al.*, *J. Cell Sci.* **119**, 797-806 (2006)
- 5) Tsunoda, K., *et al.*, *J. Immunol.* **170**, 2170-2178 (2003)

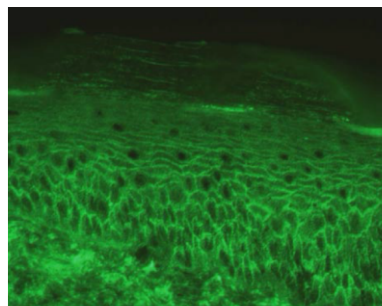
Clone AK23 is used in this reference.

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Immunohistochemical detection of Dsg3 on frozen section of normal human skin with D219-3.



Immunohistochemical detection of Dsg3 on frozen section of mouse oral mucosa with D219-3.

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

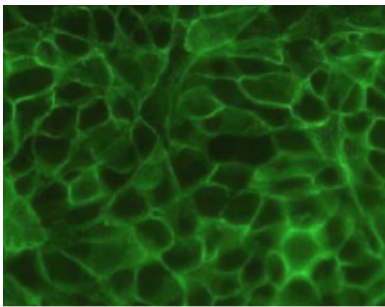
The descriptions of the following protocols are examples.
Each user should determine the appropriate condition.

PROTOCOLS:

Immunohistochemical staining for frozen sections

- 1) Cover tissues with primary antibody 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).
- 4) Wipe gently around each section and cover tissues with FITC conjugated anti-mouse IgG antibody 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).
- 7) Now ready for mounting.

(Positive controls for Immunohistochemistry; Human skin and mouse oral mucosa)



Immunocytochemical detection of Dsg3 on living mouse keratinocyte cell line (PAM212 cells) with D219-3.

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)

Immunocytochemistry for living keratinocyte

- 1) Prepare 80-90% confluent mouse keratinocyte cells in chamber slide.
- 2) Wash the cells twice 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).
- 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).
- 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).

- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; PAM212)