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Datasheet for 600-401-GN5

# JAM A phospho Y280 Antibody

## Overview

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|---|---|
| **Description:** | Anti-JAM A pY280 (RABBIT) Antibody - 600-401-GN5 |
| **Item No.:** | 600-401-GN5 |
| **Size:** | 100 µg |
| **Applications:** | ELISA, IF, WB, Multiplex |
| **Reactivity:** | Human |
| **Host Species:** | Rabbit |

## Product Details

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| **Background:** | Anti-JAM A pY280 was designed, produced, and validated as part of the Joy Cappel Young Investigator Award (JCYIA). JAM A pY280 Antibody detects junctional adhesion molecule A (JAM A) protein phosphorylated at the tyrosine 280 location. JAM A is involved in cell-cell contact and tight junction formation in epithelial cells. JAM A protein is a transmembrane protein expressed in epithelial and endothelial cells as well as in hematopoietic cells and is important for a variety of cellular processes including tight junction assembly, leukocyte transmigration, platelet activation, angiogenesis and virus binding. In addition, JAM A may be a potential therapeutic target for breast cancer. |
| **Synonyms:** | rabbit anti-JAM A pY280 antibody, JAM-A, Junctional adhesion molecule A, JAM-1, Junctional adhesion molecule 1, Platelet F11 receptor, Platelet adhesion molecule 1, PAM-1, CD321, JAM1, JCAM, JAM 1, JAMA |
| **Host Species:** | Rabbit |
| **Clonality:** | Polyclonal |
| **Format:** | IgG |

## Target Details

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| **Gene Name:** | F11R |
| **Reactivity:** | Human |
| **PTM Specificity:** | Phosphorylation |
| **Immunogen Type:** | Conjugated Peptide |

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| **Immunogen:** | Affinity purified Anti-JAM A pY280 antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to the c-term and phosphorylated at the tyrosine 280 position of Human JAM A protein. |
| **Purity/Specificity:** | Anti-JAM A pY280 is directed against human JAM A phosphorylated at the tyrosine 280 position. This product is an affinity purified antibody produced by immunoaffinity chromatography using phospho peptide coupled to agarose beads followed by solid phase adsorption(s) against non-phospho peptide to remove any unwanted reactivities. A BLAST analysis was used to suggest reactivity with this protein from human and feline based on 100% homology for the immunogen sequence. |
| **Relevant Links:** | • UniProtKB - Q9Y624<br>• NCBI - NP_058642.1<br>• GENEID - 50848 |

## Application Details

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| **Tested Applications:** | ELISA, IF, WB |
| **Suggested Applications:** | Multiplex (Based on references) |
| **Application Note:** | This affinity purified antibody has been tested for use in ELISA, IF, and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band ~ 32.5 kDa in size corresponding to JAM A by western blotting in the appropriate cell lysate or extract. |
| **Assay Dilutions:** | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below. |
| **ELISA:** | 5 ug/ml |
| **IF:** | User optimized |
| **WB:** | 1 ug/ml |

## Formulation

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| **Physical State:** | Liquid (sterile filtered) |
| **Concentration:** | 1.02mg/mL by UV absorbance at 280 nm |
| **Buffer:** | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| **Preservative:** | 0.01% (w/v) Sodium Azide |
| **Stabilizer:** | None |

## Shipping & Handling

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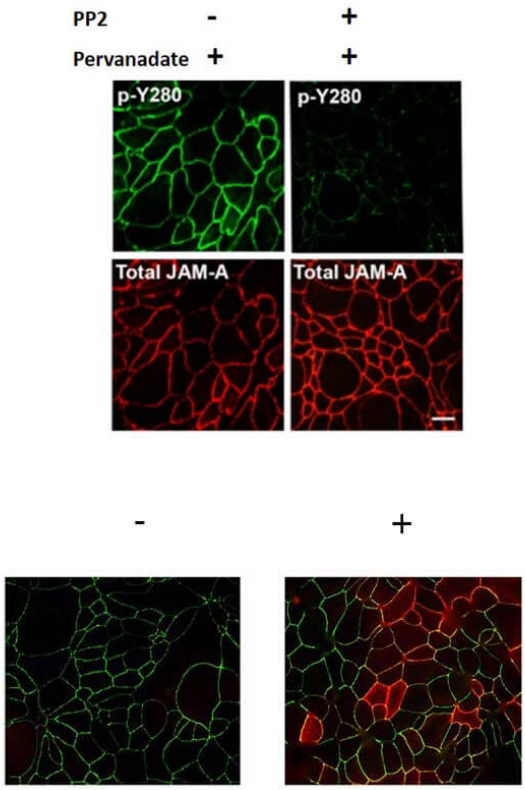
| **Shipping Condition:** | Dry Ice |
| **Storage Condition:** | Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. |
| **Expiration:** | Expiration date is one (1) year from date of receipt. |

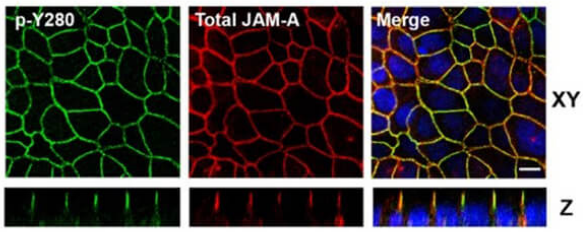
## Images



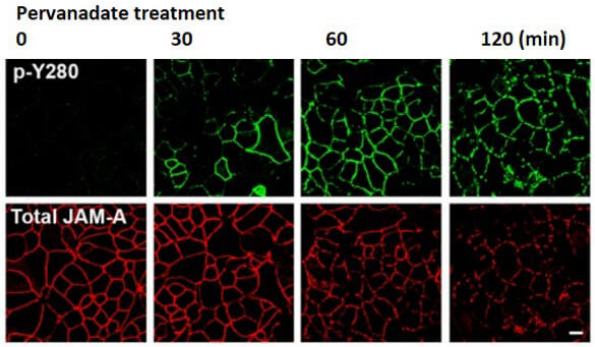

**Immunofluorescence Microscopy**
Immunofluorescence Microscopy of Rabbit Anti-JAM-A pY280 antibody. Tissue: T84 cells. Pretreatment: PP2. Treatment: Pervanadate. Fixation: 4% PFA. Permeabilization: 1% SDS. Costained Green: Anti-Phospho JAM-A Y280 Antibody, FITC conjugated secondary; Red: Anti-Total JAM-A, Alexa-conjugated secondary antibodies. Results: The Src family kinase inhibitor PP2 inhibits pervanadate-dependent phosphorylation of JAM-A Y280, as they reported to modulate tyrosine phosphorylation of junctional proteins and influence epithelial barrier function. See additional information in Mol Biol Cell. 2019 Mar 1; 30 (5): 566–578. PMID: 30625033.



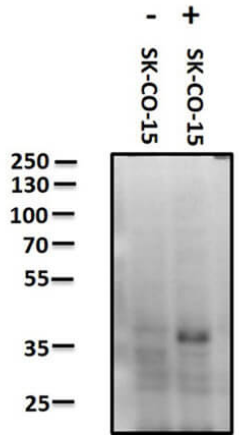

**Immunofluorescence Microscopy**
Immunofluorescence Microscopy of Rabbit anti-JAMA pY280 antibody. Tissue: T84 cells (untreated/treated). Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: JAMA pY280 antibody at 2 µg/mL for 1 hr at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1:10,000 for 45 min at RT. Localization: JAMA pY280 is along the cell membrane and cell junction. Staining: JAMA pY280 as red fluorescent signal. JCYIA



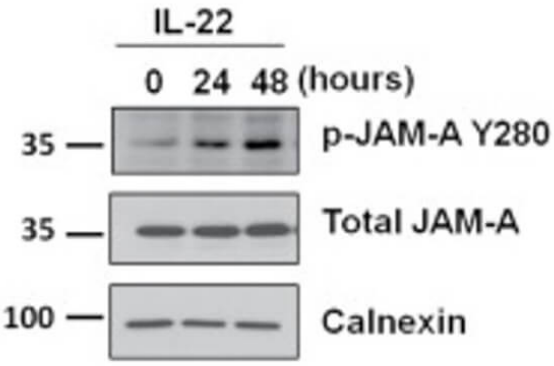

**Immunofluorescence Microscopy**
Confocal Immunofluorescence Microscopy of Rabbit Anti-JAM-A pY280 antibody in polarized epithelial cells. Tissue: T84 cells were grown on Transwell filters until confluent. Treatment: pervanadate. Fixation: 4% PFA. Permeabilization: 1% SDS. Costained Green: Anti-Phospho JAM-A Y280 Antibody, FITC conjugated secondary; Red: Anti-Total JAM-A, Alexa-conjugated secondary antibodies. Localization: tight junctions, seen in Confocal Z-stacks. Scale bar: 10 µm. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.



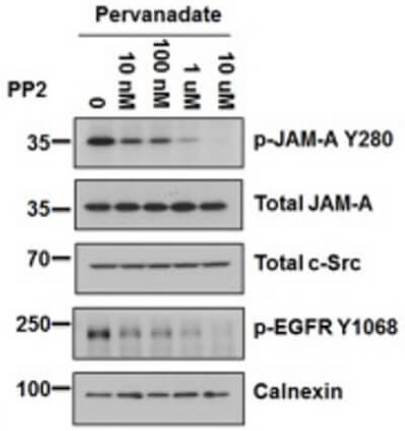

**Immunofluorescence Microscopy**
Confocal Immunofluorescence Microscopy of Rabbit Anti-JAM-A pY280 antibody of confluent (intestinal epithelial cells) IECs. Tissue: SK CO-15 cells. Treatment: pervanadate at time points 0, 30, 60, 120 mins. Fixation: 4% PFA. Permeabilization: 1% SDS. Costained Green: Anti-Phospho JAM-A Y280 Antibody, FITC conjugated secondary; Red: Anti-Total JAM-A, Alexa-conjugated secondary antibodies. Results: pervanadate treatment led to a time-dependent increase in phosphorylation of JAM-A Y280 that correlated with decreased localization of JAM-A at cell–cell contacts. Scale bar: 10 µm. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.




**Western Blot**
Western Blot of Rabbit anti-JAM A pY280 antibody. Lane 1: SK-CO-15 negative control. Lane 2: SK-CO-15 pervanadate treated positive control. Load: 10 µg per lane. Primary antibody: JAM A pY280 antibody at 1 ug/mL for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody at 1:40,000 for 30 min at RT. Block: MB-070 for 30 minutes at RT. Predicted/Observed size: ~ 32.5 kDa. JCYIA




**Western Blot**
Western Blot of Rabbit Anti-JAM-A pY280 antibody with IL-22. Lysates: T84 cells. Treatment: hu rec. IL-22 at time points 0, 24, 48 hrs. Primary antibodies: JAM-A pY280, total JAM-A, or Calnexin. Calnexin was used as a loading control. Secondary antibody: horseradish peroxidase secondary antibody. Results: Exposure of IECs to other cytokines (IL-17A, IL-22, TNFα, or IFNγ) results in tyrosine phosphorylation of JAM-A at Y280 and a leaky barrier. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.




**Western Blot**
Western Blot of Rabbit Anti-JAM-A pY280 antibody with PP2. Lysates: T84 cells. Treatments: Pervanadate; PP2 at 0, 10nM, 100nM, 1µM, 10µM. Primary antibodies: p-JAM-A Y280, total JAM-A, total c-Src, p-EGFR Y1068, or Calnexin. p-EGFR Y1068 was used as a positive control for PP2. Calnexin was used as a loading control. Secondary antibody: horseradish peroxidase secondary antibody. Results: PP2 dose-dependent decrease in tyrosine phosphorylation of JAM-A Y280 following pervanadate treatment. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.

## References


- Tapia R, Kralicek SE, Hecht GA. Enteropathogenic Escherichia coli (EPEC) Recruitment of PAR Polarity Protein Atypical PKCζ to Pedestals and Cell-Cell Contacts Precedes Disruption of Tight Junctions in Intestinal Epithelial Cells. *Int J Mol Sci.* (2020)
- Fan S., Parkos C. et al. Role of JAM-A tyrosine phosphorylation in epithelial barrier dysfunction during intestinal inflammation. *Molecular Biology of the Cell* (2019)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