

Datasheet for 600-401-GN5S**JAM A phospho Y280 Antibody****Overview**

Description:	Anti-JAM A pY280 (RABBIT) Antibody - 600-401-GN5S
Item No.:	600-401-GN5S
Size:	25 µL
Applications:	ELISA, IF, WB, Multiplex
Reactivity:	Human
Host Species:	Rabbit

Product Details

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

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

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 • NCBI - NP_058642.1 • GENEID - 50848

Application Details

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

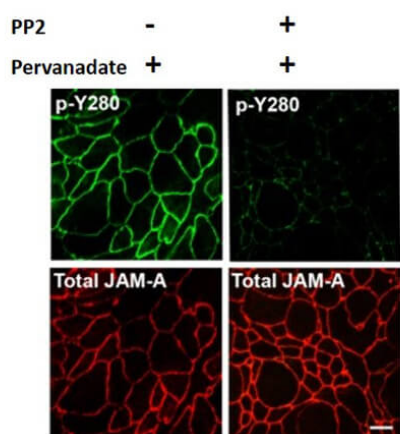
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/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

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

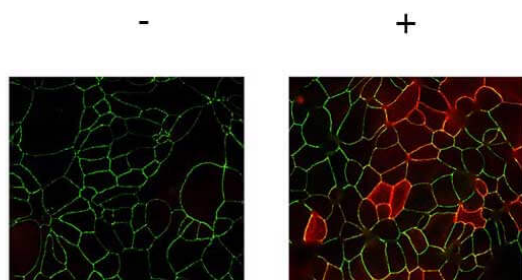


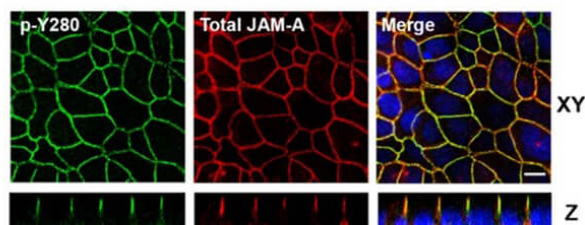
Immunofluorescence Microscopy

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

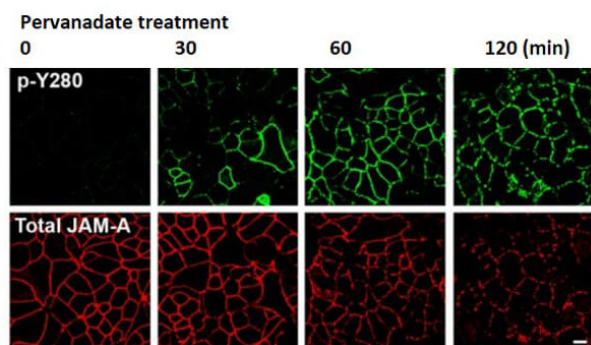
Immunofluorescence Microscopy of Rabbit anti-JAMA pY280 antibody. Tissue: T84 cells (untreated/treated). Fixation: 0.5% PFA. Antigen retrieval: not required. Primary antibody: JAMA pY280 antibody at 2 µg/mL for 1 hr at RT. Secondary antibody: Fluorescein rabbit secondary antibody at 1:10,000 for 45 min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





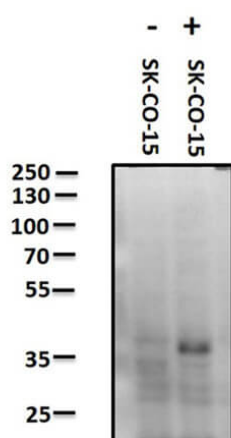
Immunofluorescence Microscopy

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 μ m. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.



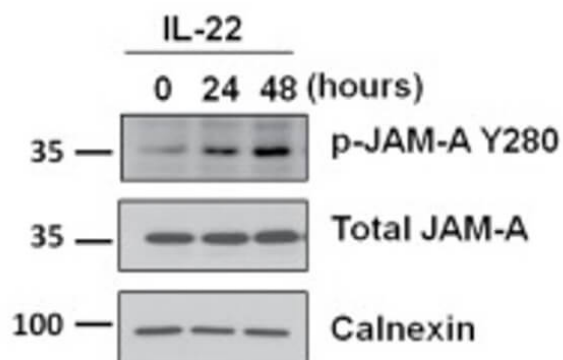
Immunofluorescence Microscopy

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 μ m. See additional information in Mol Biol Cell. 2019 Mar 1; 30(5): 566–578. PMID: 30625033.



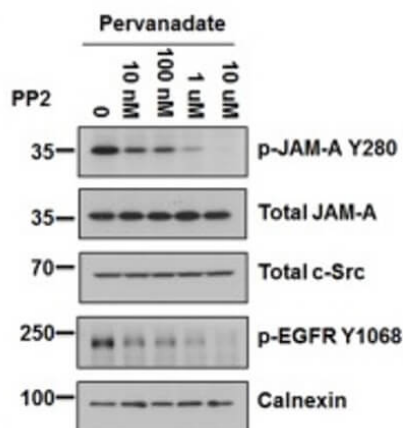
Western Blot

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 μ g per lane. Primary antibody: JAM A pY280 antibody at 1 μ g/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, TNF α , or IFN γ) 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, 1 μ M, 10 μ M. 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 PKC ζ 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)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.