10320 Camino Santa Fe, Suite G
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Tel: 858.875.1900 Fax: 858.875.1999

MICA Antibody (Center)

Affinity Purified Rabbit Polyclonal Antibody (Pab)
Catalog # AP8626c

Specification

MICA Antibody (Center) - Product Information

Application	**IF, WB, IHC-P, FC,E**
Primary Accession	Q29983
Reactivity	**Human**
Host	**Rabbit**
Clonality	**Polyclonal**
Isotype	**Rabbit IgG**
Antigen Region	**68-97**

MICA Antibody (Center) - Additional Information

Gene ID 100507436

Other Names
MHC class I polypeptide-related sequence A, MIC-A, MICA {ECO:0000312|EMBL:CAI419071}

Target/Specificity
This MICA antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 68-97 amino acids from the Central region of human MICA.

Dilution
IF~~1:25
WB~~1:2000
IHC-P~~1:25
FC~~1:25

Format
Purified polyclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein A column, followed by peptide affinity purification.

Storage
Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

Precautions
MICA Antibody (Center) is for research use only and not for use in diagnostic or therapeutic procedures.

MICA Antibody (Center) - Protein Information

Name MICA {ECO:0000312|EMBL:CAI41907.1}

Function Widely expressed membrane-bound protein which acts as a ligand to stimulate an

activating receptor KLRK1/NKG2D, expressed on the surface of essentially all human natural killer (NK), gammadelta T and CD8 alphabeta T-cells (PubMed:11491531, PubMed:11777960). Up-regulated in stressed conditions, such as viral and bacterial infections or DNA damage response, serves as signal of cellular stress, and engagement of KLRK1/NKG2D by MICA triggers NK-cells resulting in a range of immune effector functions, such as cytotoxicity and cytokine production (PubMed:10426993).

Cellular Location
Cell membrane; Single-pass type I membrane protein. Cytoplasm Note=Expressed on the cell surface in gastric epithelium, endothelial cells and fibroblasts and in the cytoplasm in keratinocytes and monocytes. Infection with human adenovirus 5 suppresses cell surface expression due to the adenoviral E3-19K protein which causes retention in the endoplasmic reticulum.

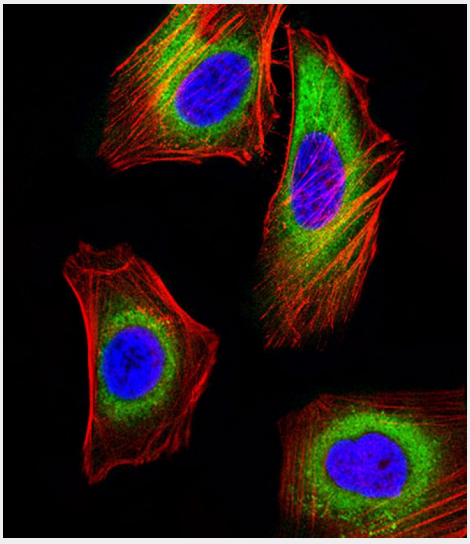
Tissue Location
Widely expressed with the exception of the central nervous system where it is absent. Expressed predominantly in gastric epithelium and also in monocytes, keratinocytes, endothelial cells, fibroblasts and in the outer layer of Hassal's corpuscles within the medulla of normal thymus. In skin, expressed mainly in the keratin layers, basal cells, ducts and follicles. Also expressed in many, but not all, epithelial tumors of lung, breast, kidney, ovary, prostate and colon. In thyomas, overexpressed in cortical and medullar epithelial cells. Tumors expressing MICA display increased levels of gamma delta T-cells.

MICA Antibody (Center) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

MICA Antibody (Center) - Images



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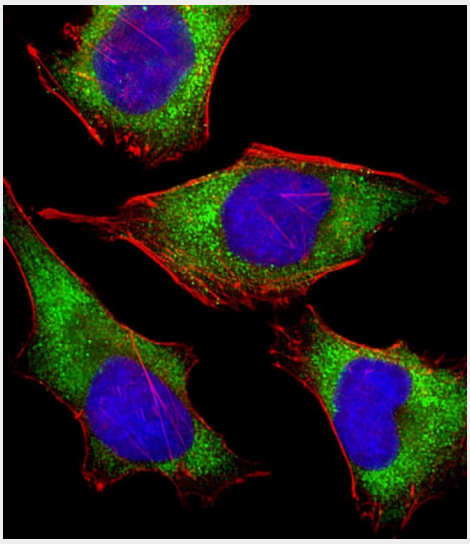
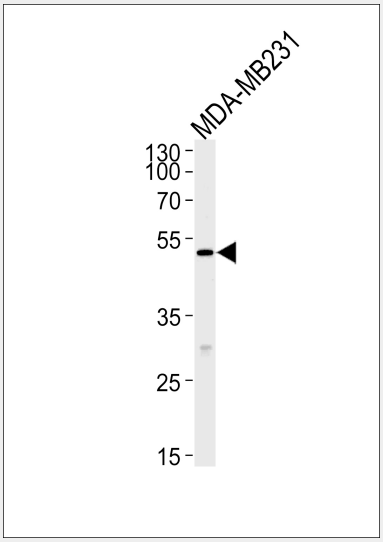
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Hela (Human Cervical epithelial adenocarcinoma cell line) cells labeling MICA with AP8626c at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-rabbit IgG (NK179883) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm staining on Hela cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).The nuclear counter stain is DAPI (blue).



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling MICA with AP8626c at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-rabbit IgG (NK179883) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).The nuclear counter stain is DAPI (blue).



MICA Antibody (Center) (Cat.# AP8626c) western blot analysis in MDA-MB-231 cell line lysates (35ug/lane).This demonstrates the MICA antibody detected the MICA protein (arrow).

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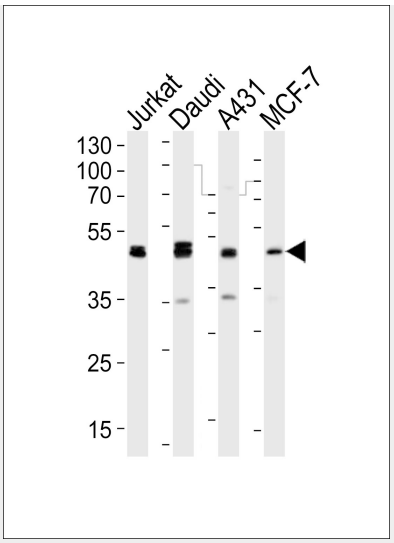
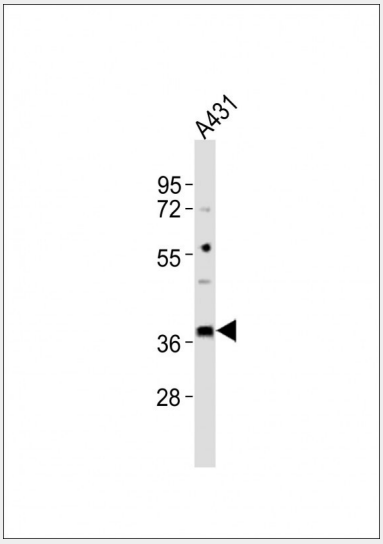


Western blot analysis of lysates from Jurkat, Daudi, A431, MCF-7 cell line (from left to right), using MICA Antibody (Center)(Cat. #AP8626c). AP8626c was diluted at 1:1000 at each lane. A goat anti-rabbit IgG H&L(HRP) at 1:10000 dilution was used as the secondary antibody. Lysates at 20ug per lane.



Anti-MICA Antibody (Center)at 1:2000 dilution + A431 whole cell lysates Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution Predicted band size : 43 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

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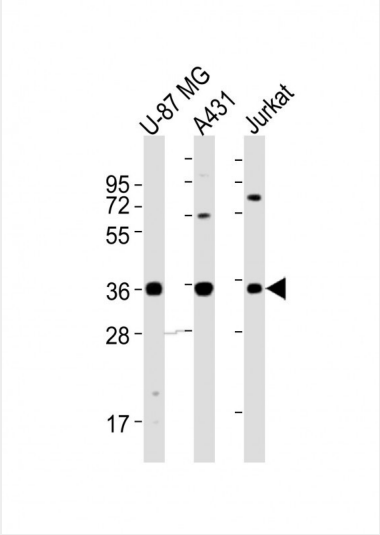
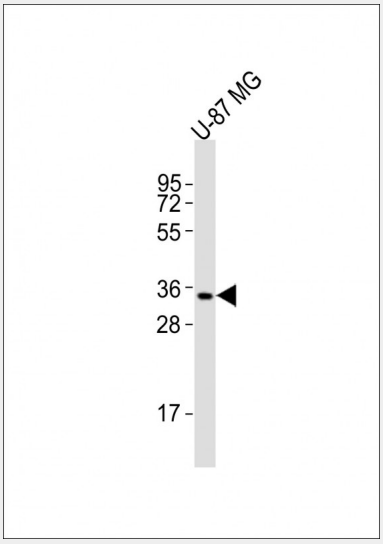


All lanes : Anti-MICA Antibody (Center) at 1:2000 dilution Lane 1: U-87 MG whole cell lysates Lane 2: A431 whole cell lysates Lane 3: Jurkat whole cell lysates Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution Predicted band size : 43 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-MICA Antibody (Center)at 1:2000 dilution + U-87 MG whole cell lysates Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution Predicted band size : 43 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

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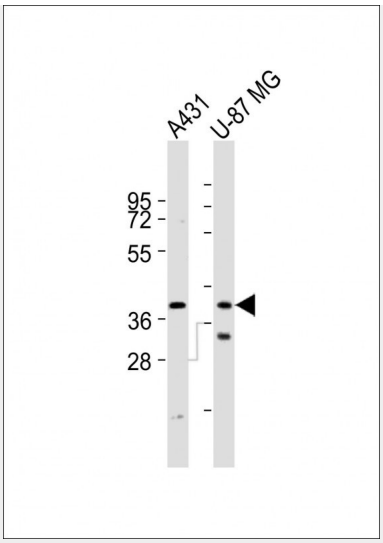
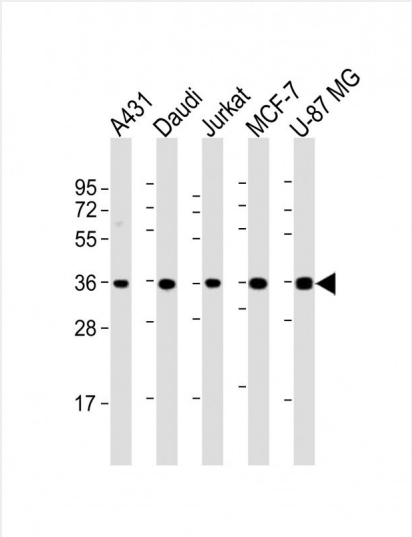


All lanes : Anti-MICA Antibody (Center) at 1:2000 dilution Lane 1: A431 whole cell lysates Lane 2: U-87 MG whole cell lysates Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution Predicted band size : 43 kDa Blocking/Dilution buffer: 5% NFDM/TBST.



All lanes : Anti-MICA Antibody (Center) at 1:2000 dilution Lane 1: A431 whole cell lysate Lane 2: Daudi whole cell lysate Lane 3: Jurkat whole cell lysate Lane 4: MCF-7 whole cell lysate Lane 5: U-87 MG whole cell lysate Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 43 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

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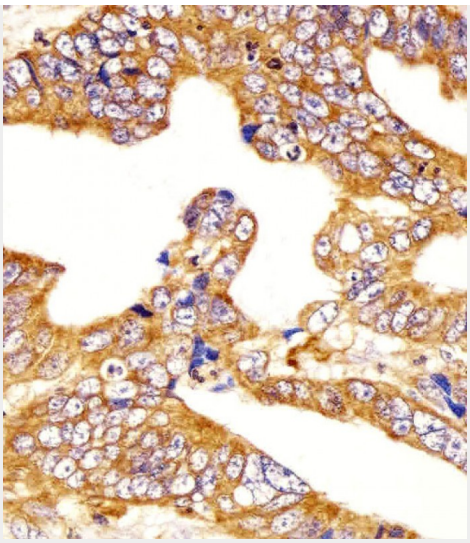
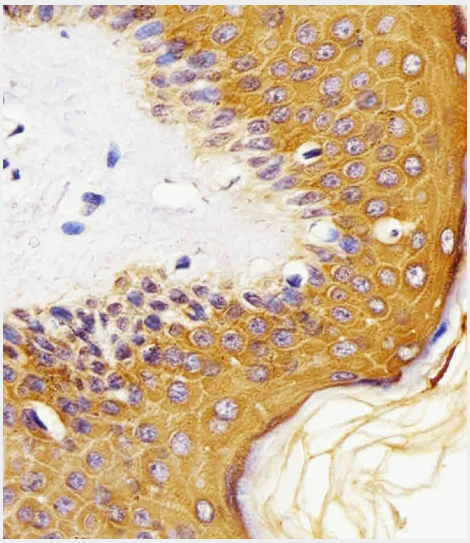


Immunohistochemical analysis of paraffin-embedded H. colorectal carcinoma section using MICA Antibody (Center)(Cat#AP8626C). AP8626C was diluted at 1:25 dilution. A undiluted biotinylated goat polyvalent antibody was used as the secondary, followed by DAB staining.



AP8626c staining MICA in Human skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.

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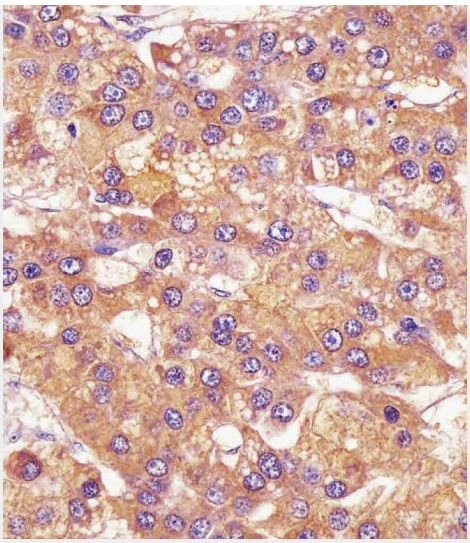
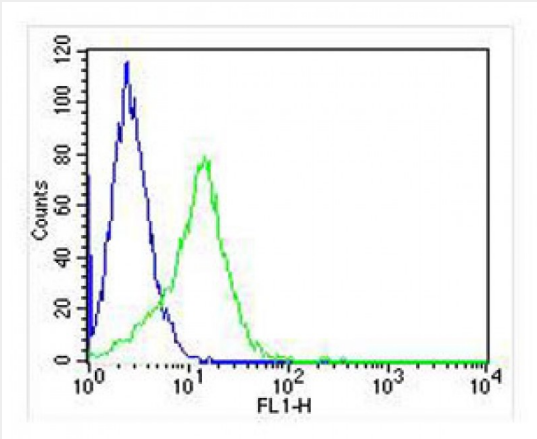


AP8626c staining MICA in human hepatic carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.



Overlay histogram showing SK-BR-3 cells stained with AP8626c (green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AP8626c, 1:25 dilution) for 60 min at 37ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (1583138) at 1/400 dilution for 40 min at 37ºC. Isotype control antibody (blue line) was rabbit IgG1 (1µg/1x10^6 cells) used under the same conditions. Acquisition of >10, 000 events was performed.

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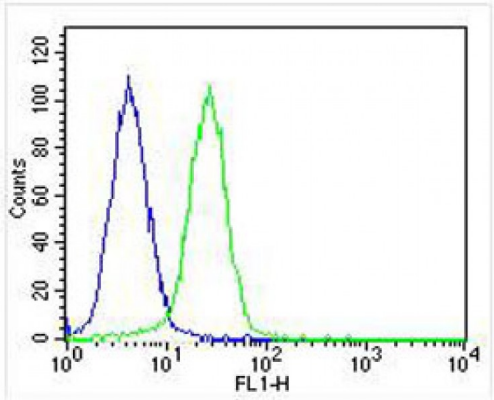

Overlay histogram showing Hela cells stained with AP8626c (green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AP8626c, 1:25 dilution) for 60 min at 37ºC. The secondary antibody used was Goat-Anti-Rabbit IgG, DyLight® 488 Conjugated Highly Cross-Adsorbed(OH191631) at 1/400 dilution for 40 min at 37ºC. Isotype control antibody (blue line) was rabbit IgG (1µg/1x10^6 cells) used under the same conditions. Acquisition of >10, 000 events was performed.

MICA Antibody (Center) - Background

MICA is the higly polymorphic MHC (HLA) class I chain-related gene A. The protein product is expressed on the cell surface, although unlike canonical class I molecules does not seem to associate with beta-2-microglobulin. It is thought that MICA functions as a stress-induced antigen that is broadly recognized by intestinal epithelial gamma delta T cells.

MICA Antibody (Center) - References

Bahram,S., et.al., Proc. Natl. Acad. Sci. U.S.A. 91 (14), 6259-6263 (1994) Klein,J.et.al., Proc. Natl. Acad. Sci. U.S.A. 91 (14), 6251-6252 (1994) Parham,P., et.al., J. Immunol. 142 (11), 3937-3950 (1989)

MICA Antibody (Center) - Citations

- Allele Specific Expression of MICA Variants in Human Fibroblasts Suggests a Pathogenic Mechanism.
- 2-deoxy D-glucose prevents cell surface expression of NKG2D ligands through inhibition of N-linked glycosylation.