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Anti-Cytokeratin 8 KRT8 Antibody Picoband™ (monoclonal, 3G9)

Catalog # ABO14840

Product Information

Application	WB, IHC, IHC-F, IF, ICC, FC
Primary Accession	P05787
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Cytokeratin 8 KRT8 Antibody Picoband™ (monoclonal, 3G9) . Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3856
Other Names	Keratin, type II cytoskeletal 8, Cytokeratin-8, CK-8, Keratin-8, K8, Type-II keratin Kb8, KRT8, CYK8
Calculated MW	53704
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunohistochemistry (Frozen Section), 0.5-1 □g/ml Immunofluorescence, 2 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Nucleoplasm. Nucleus matrix. Cytoplasm.
Tissue Specificity	Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 3G9
Immunogen	E.coli-derived human Cytokeratin 8 recombinant protein (Position:

	D107-K325). Human Cytokeratin 8 shares 95.4% and 94.5% amino acid (aa) sequence identity with mouse and rat Cytokeratin 8, respectively.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	KRT8 (HGNC:6446)
Synonyms	CYK8
Function	Structural component of intermediate filaments in simple epithelial cells. Together with its obligate type I partner KRT18, contributes to the formation of the keratin intermediate filament network that provides mechanical stability and resilience to epithelial cells (PubMed:26823019). Also involved in maintaining epithelial integrity and protecting cells from non-mechanical stress, such as apoptosis (By similarity). Also required for ARHGEF40-mediated actin stress fiber formation and tensional force-induced stress fiber formation and reinforcement (PubMed:26823019). In striated muscle, together with KRT19, may contribute to linking the contractile apparatus to dystrophin at the costameres (PubMed:16000376).
Cellular Location	Cytoplasm. Nucleus, nucleoplasm {ECO:0000250|UniProtKB:Q10758}. Nucleus matrix {ECO:0000250|UniProtKB:Q10758}. Cytoplasm, cytoskeleton
Tissue Location	Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.

Background

Keratin, type II cytoskeletal 8, also known as cytokeratin-8 (CK-8) or keratin-8 (K8) is a keratin protein that is encoded in humans by the KRT8 gene. This gene is a member of the type II keratin family clustered on the long arm of chromosome 12. Type I and type II keratins heteropolymerize to form intermediate-sized filaments in the cytoplasm of epithelial cells. The product of this gene typically dimerizes with keratin 18 to form an intermediate filament in simple single-layered epithelial cells. This protein plays a role in maintaining cellular structural integrity and also functions in signal transduction and cellular differentiation. Mutations in this gene cause cryptogenic cirrhosis. Alternatively spliced transcript variants have been found for this gene.

Images

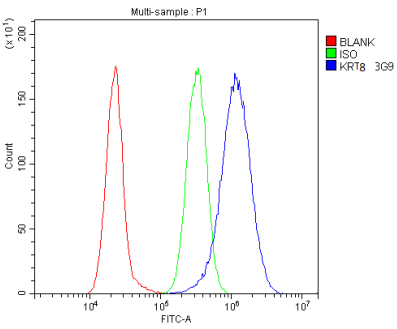


Figure 1. Flow Cytometry analysis of A549 cells using anti-Cytokeratin 8 antibody (M01421-3).
Overlay histogram showing A549 cells stained with M01421-3 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Cytokeratin 8 Antibody (M01421-3, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6)

used under the same conditions. Unlabelled sample (Red line) was also used as a control.

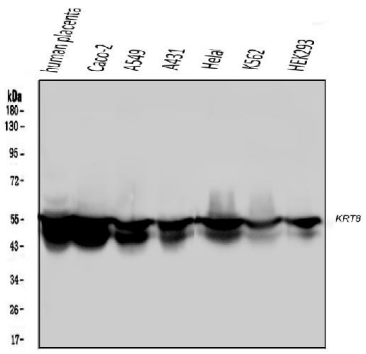


Figure 2. Western blot analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody (M01421-3).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human placenta tissue lysates;
Lane 2: human Caco-2 whole cell lysates;
Lane 3: human A549 whole cell lysates;
Lane 4: human A431 whole cell lysates;
Lane 5: human Hela whole cell lysates;
Lane 6: human K562 whole cell lysates;
Lane 7: human HEK293 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Cytokeratin 8 antigen affinity purified monoclonal antibody (Catalog # M01421-3) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Cytokeratin 8 at approximately 54KD. The expected band size for Cytokeratin 8 is at 54KD.

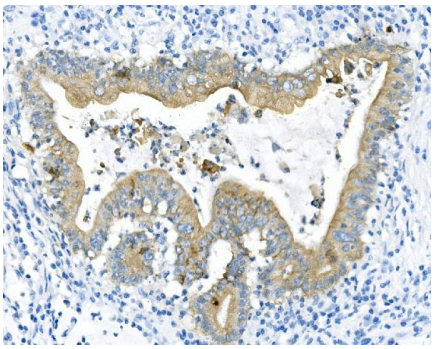


Figure 3. IHC analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody (M01421-3).
Cytokeratin 8 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Cytokeratin 8 Antibody (M01421-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-Mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

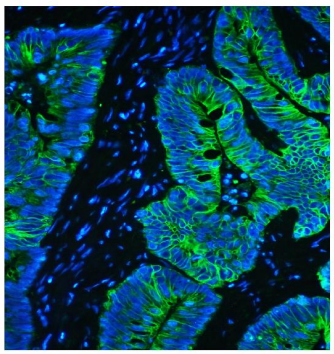


Figure 4. IF analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody (M01421-3).
Cytokeratin 8 was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-Cytokeratin 8 Antibody (M01421-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

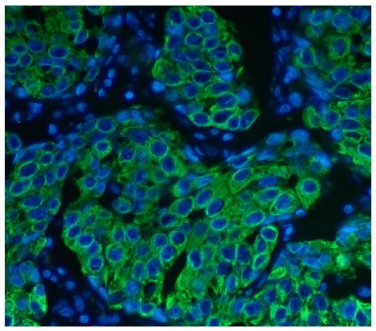


Figure 5. IF analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody (M01421-3). Cytokeratin 8 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/mL mouse anti-Cytokeratin 8 Antibody (M01421-3) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

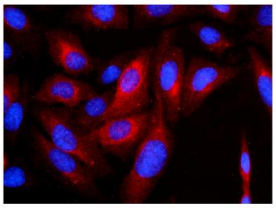


Figure 6. IF analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody (M01421-3). Cytokeratin 8 was detected in immunocytochemical section of U20S cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/ml mouse anti-Cytokeratin 8 Antibody (M01421-3) overnight at 4°C. Biotin Conjugated Goat anti-Mouse IgG (BA1001) was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Cy3 Conjugated Avidin (BA1037). Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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