Product Datasheet

Cytokeratin 8 KRT8 Antibody (monoclonal, 3G9) (orb527059)

Catalog Number	orb527059
Category	Antibodies
Description	Anti-Cytokeratin 8 KRT8 Antibody (monoclonal, 3G9). Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Clonality	Monoclonal
Species/Host	Mouse
Isotype	Mouse IgG2b
Conjugation	Unconjugated
Reactivity	Human, Mouse, Rat
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Purification	Immunogen affinity purified.
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.
UniProt ID	P05787
MW	54 kDa
Tested applications	FC, ICC, IF, IHC, IHC-Fr, WB

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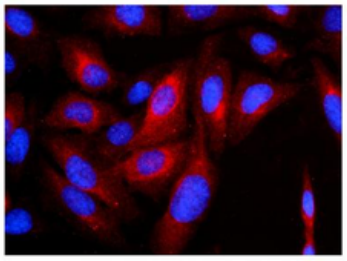
Application notes	Western blot, 0.1-0.5µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml Immunohistochemistry (Frozen Section), 0.5-1µg/ml Immunofluorescence, 2µg/ml Immunocytochemistry/Immunofluorescence, 2µg/ml Flow Cytometry (Fixed), 1-3µg/1x106 cells. Add 0.2ml of distilled water will yield a concentration of 500µg/ml
Cross Reactivity	No cross-reactivity with other proteins.
Antibody Type	Primary Antibody
Clone Number	3G9
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.
Note	For research use only



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

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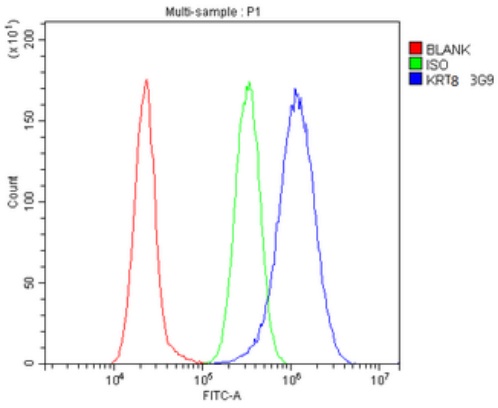
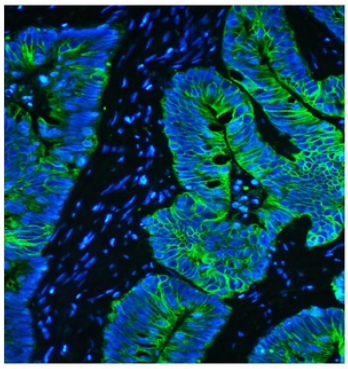
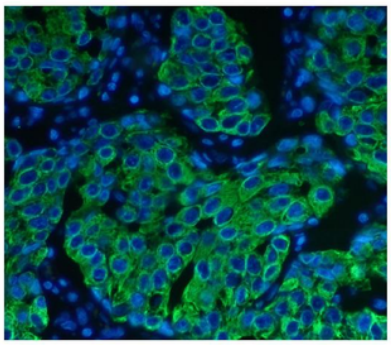


Flow Cytometry analysis of A549 cells using anti-Cytokeratin 8 antibody. Overlay histogram showing A549 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Cytokeratin 8 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (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.



IF analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody. 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 overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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.



IF analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody. 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 overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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.

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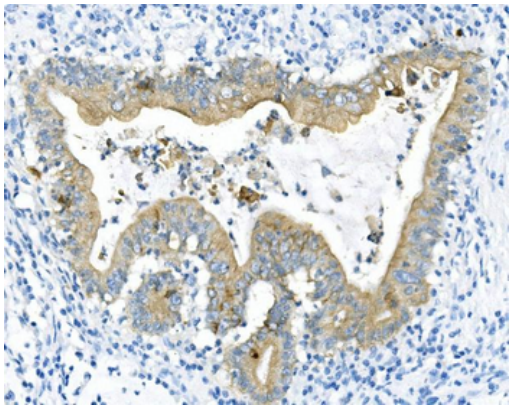
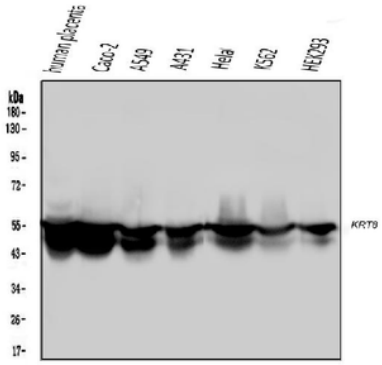


IHC analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody. Cytokeratin 8 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Cytokeratin 8 Antibody 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 with DAB as the chromogen.



Western blot analysis of Cytokeratin 8 using anti-Cytokeratin 8 antibody. 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 50 ug 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 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 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.

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