

Anti-JAB1 Antibody Picoband™ (monoclonal, 4G9)

Catalog # ABO15061

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q92905
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-JAB1 Antibody Picoband™ (monoclonal, 4G9) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	10987
Other Names	COP9 signalosome complex subunit 5, SGN5, Signalosome subunit 5, 3.4.-.-, Jun activation domain-binding protein 1, COPS5, CSN5, JAB1
Calculated MW	37579
Application Details	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na ₂ HPO ₄ .
Clone Names Immunogen	Clone: 4G9 E.coli-derived human JAB1 recombinant protein (Position: M8-S334). Human JAB1 shares 99.4% amino acid (aa) sequence identity with mouse JAB1.
Purification	Immunogen affinity purified.
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	COPS5
Synonyms	CSN5, JAB1
Function	Probable protease subunit of the COP9 signalosome complex (CSN), a complex involved in various cellular and developmental processes. The CSN complex is an essential regulator of the ubiquitin (Ubl) conjugation pathway by mediating the deneddylation of the cullin subunits of the SCF-type E3 ligase complexes, leading to decrease the Ubl ligase activity of SCF-type complexes such as SCF, CSA or DDB2. The complex is also involved in phosphorylation of p53/TP53, c-jun/JUN, IkappaBalpha/NFKBIA, ITPK1 and IRF8, possibly via its association with CK2 and PKD kinases. CSN-dependent phosphorylation of TP53 and JUN promotes and protects degradation by the Ubl system, respectively. In the complex, it probably acts as the catalytic center that mediates the cleavage of Nedd8 from cullins. It however has no metalloprotease activity by itself and requires the other subunits of the CSN complex. Interacts directly with a large number of proteins that are regulated by the CSN complex, confirming a key role in the complex. Promotes the proteasomal degradation of BRSK2.
Cellular Location	Cytoplasm, cytosol. Nucleus. Cytoplasm, perinuclear region. Cytoplasmic vesicle, secretory vesicle, synaptic vesicle Note=Nuclear localization is diminished in the presence of IFIT3

Background

COP9 constitutive photomorphogenic homolog subunit 5 (Arabidopsis), also known as COPS5 or JAB1, is a gene conserved from humans to *Saccharomyces cerevisiae*. It is a member of the MOV34 family. COPS5 is mapped to 8q13.1. The protein encoded by this gene is one of the eight subunits of COP9 signalosome, a highly conserved protein complex that functions as an important regulator in multiple signaling pathways. COPS5 can interact with the cytoplasmic domain of the beta-2 subunit of the alpha-L/beta-2 integrin LFA1, and it is the only protein demonstrated to interact with MIF. COPS5, VHL, and TRC8 proteins appear to be linked both physically and functionally, and all 3 may participate in the development of kidney cancer. In addition to that, COPS5 is an essential cofactor for the apoptotic function of E2F1.

Images

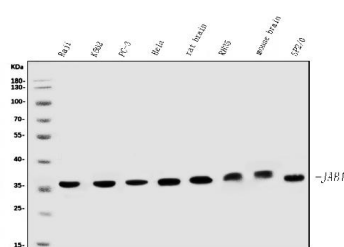


Figure 1. Western blot analysis of JAB1 using anti-JAB1 antibody (M01849-2).

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 30ug of sample under reducing conditions.

Lane 1: human Raji whole cell lysates,
Lane 2: human K562 whole cell lysates,
Lane 3: human PC-3 whole cell lysates,
Lane 4: human Hela whole cell lysates,
Lane 5: rat brain tissue lysates,
Lane 6: rat RH35 whole cell lysates,
Lane 7: mouse brain tissue lysates,
Lane 8: mouse SP2/0 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-JAB1 antigen affinity purified monoclonal antibody (Catalog # M01849-2) 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 JAB1 at approximately 37KD. The expected band size for JAB1 is at 37KD.

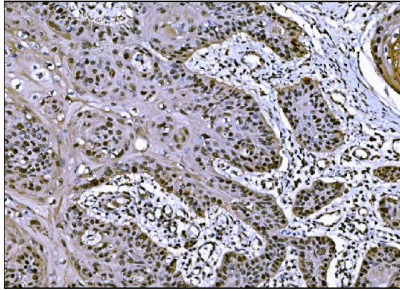


Figure 2. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2).

JAB1 was detected in paraffin-embedded section of human esophageal squamous carcinoma 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-JAB1 Antibody (M01849-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

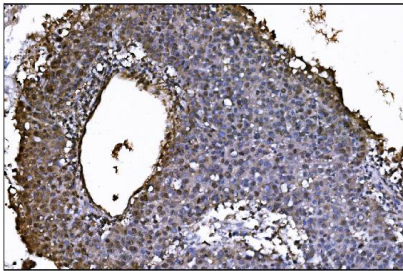


Figure 3. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2).

JAB1 was detected in paraffin-embedded section of human liver 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-JAB1 Antibody (M01849-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

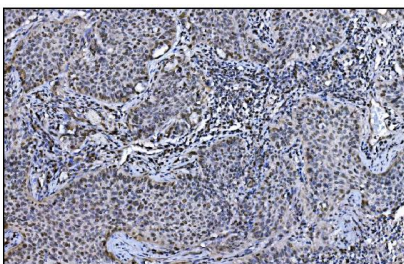
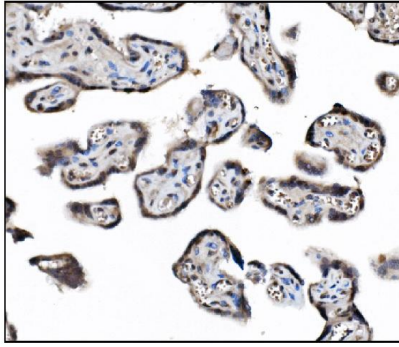


Figure 4. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2).

JAB1 was detected in paraffin-embedded section of human lung 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-JAB1 Antibody (M01849-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 5. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2).

JAB1 was detected in paraffin-embedded section of human placenta 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-JAB1 Antibody (M01849-2) overnight at



4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

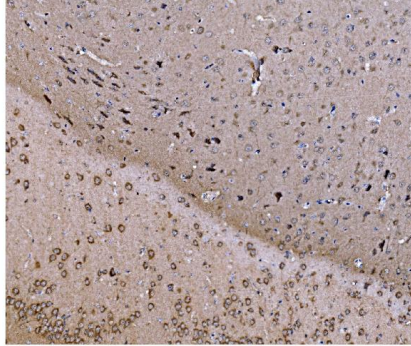


Figure 6. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2). JAB1 was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-JAB1 Antibody (M01849-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

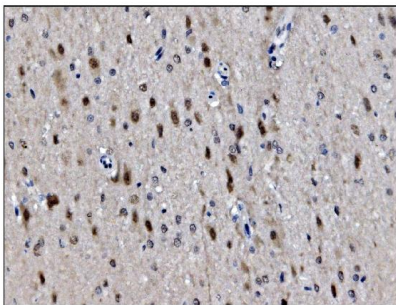


Figure 7. IHC analysis of JAB1 using anti-JAB1 antibody (M01849-2). JAB1 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-JAB1 Antibody (M01849-2) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

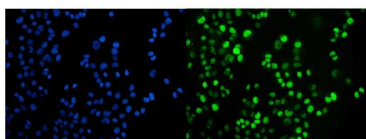
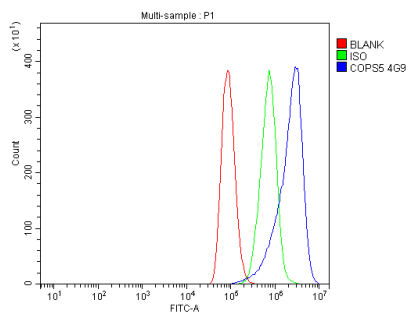


Figure 8. IF analysis of JAB1 using anti-JAB1 antibody (M01849-2). JAB1 was detected in immunocytochemical section of T-47D cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-JAB1 Antibody (M01849-2) 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 9. Flow Cytometry analysis of A549 cells using anti-JAB1 antibody (M01849-2). Overlay histogram showing A549 cells stained with M01849-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse



anti-JAB1 Antibody (M01849-2, $1 \mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C . DyLight®488 conjugated goat anti-mouse IgG (BA1126, $5\text{-}10 \mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C . Isotype control antibody (Green line) was mouse IgG ($1 \mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.