

Anti-Annexin VI Antibody Picoband™ (monoclonal, 4C4)

Catalog # ABO15088

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P08133
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Annexin VI Antibody Picoband™ (monoclonal, 4C4) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	309
Other Names	Annexin A6, 67 kDa calelectrin, Annexin VI, Annexin-6, Calphobindin-II, CPB-II, Chromobindin-20, Lipocortin VI, Protein III, p68, p70, ANXA6, ANX6
Calculated MW	75873
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 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Clone Names	Clone: 4C4
Immunogen	E. coli-derived human Annexin VI recombinant protein (Position: N395-L665).
Purification	Immunogen affinity purified.
Storage	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 freezing and thawing.

Protein Information

Name	ANXA6
Synonyms	ANX6
Function	May associate with CD21. May regulate the release of Ca(2+) from intracellular stores.
Cellular Location	Cytoplasm. Melanosome. Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV

Background

Annexin A6 (ANXA6) is a member of a family of proteins that bind membrane or cytoskeleton in a Ca(2+)-dependent manner. These proteins are characterized by homologous amino acid sequences that are present in multiple copies in each protein. ANXA6 gene is assigned to 5q32-q34 by use of a cDNA clone to probe genomic DNA from rodent-human somatic cell hybrids and for in situ hybridization. The ANX6 gene is approximately 60 kb long and contains 26 exons. The genomic sequence at the 3-prime end does not contain a canonical polyadenylation signal. Ca(2+)-dependent binding between CRHSP28 and ANXA6 is required for acinar cell membrane trafficking events and digestive enzyme secretion.

Images

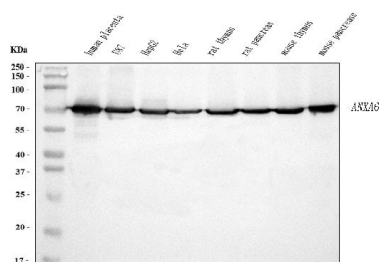


Figure 1. Western blot analysis of Annexin VI using anti-Annexin VI antibody (M03735-1).

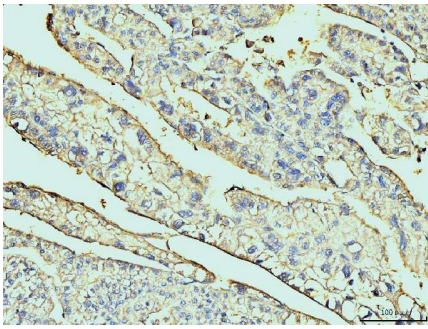
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human placenta tissue lysates,
 Lane 2: human U87 whole cell lysates,
 Lane 3: human HepG2 whole cell lysates,
 Lane 4: human Hela whole cell lysates,
 Lane 5: rat thymus tissue lysates,
 Lane 6: rat pancreas tissue lysates,
 Lane 7: mouse thymus tissue lysates,
 Lane 8: mouse pancreas tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA 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-Annexin VI antigen affinity purified monoclonal antibody (Catalog # M03735-1) 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 Annexin VI at approximately 72 kDa. The expected band size for Annexin VI is at 72 kDa.

Figure 2. IHC analysis of Annexin VI using anti-Annexin VI antibody (M03735-1).

Annexin VI was detected in a paraffin-embedded section of human liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat



serum. The tissue section was then incubated with 2 $\mu\text{g/ml}$ mouse anti-Annexin VI Antibody (M03735-1) 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.

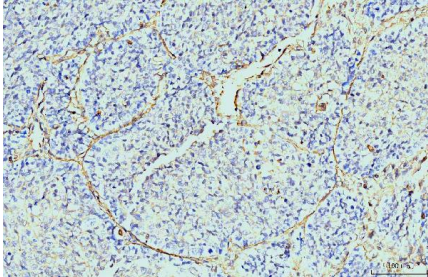


Figure 3. IHC analysis of Annexin VI using anti-Annexin VI antibody (M03735-1).

Annexin VI was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 $\mu\text{g/ml}$ mouse anti-Annexin VI Antibody (M03735-1) 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.

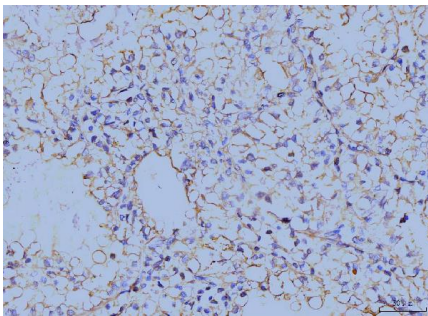


Figure 4. IHC analysis of Annexin VI using anti-Annexin VI antibody (M03735-1).

Annexin VI was detected in a paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 $\mu\text{g/ml}$ mouse anti-Annexin VI Antibody (M03735-1) 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.

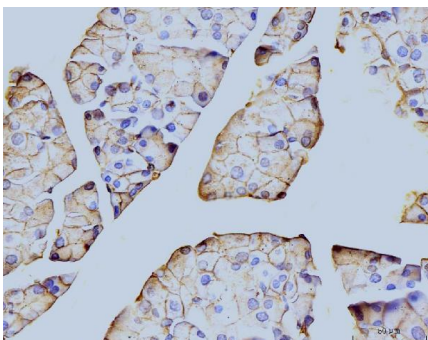
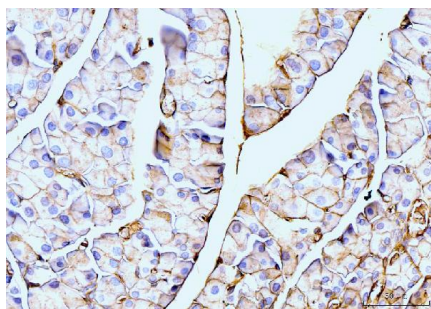


Figure 5. IHC analysis of Annexin VI using anti-Annexin VI antibody (M03735-1).

Annexin VI was detected in a paraffin-embedded section of mouse pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 $\mu\text{g/ml}$ mouse anti-Annexin VI Antibody (M03735-1) 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 Annexin VI using anti-Annexin VI antibody (M03735-1).

Annexin VI was detected in a paraffin-embedded section of rat pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2



$\mu\text{g/ml}$ mouse anti-Annexin VI Antibody (M03735-1) 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.

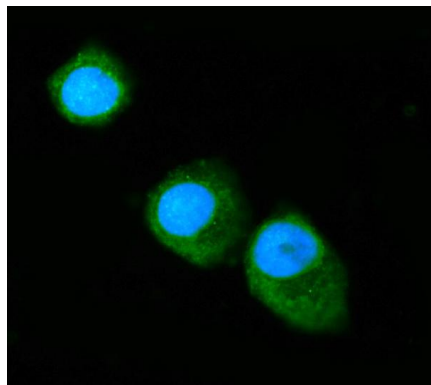


Figure 7. IF analysis of Annexin VI using anti-Annexin VI antibody (M03735-1). Annexin VI was detected in an immunocytochemical section of T-47D cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with $5\text{ }\mu\text{g/mL}$ mouse anti-Annexin VI Antibody (M03735-1) 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.

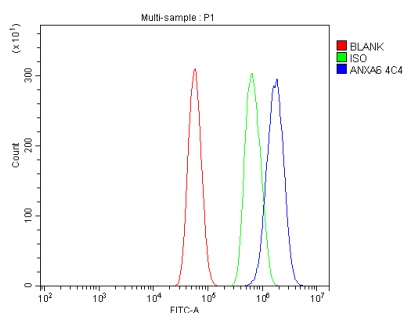


Figure 8. Flow Cytometry analysis of U87 cells using anti-Annexin VI antibody (M03735-1). Overlay histogram showing U87 cells stained with M03735-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Annexin VI Antibody (M03735-1, $1\text{ }\mu\text{g}/1\times 10^6$ cells) for 30 min at 20°C . DyLight®488 conjugated goat anti-mouse IgG (BA1126, $5\text{--}10\text{ }\mu\text{g}/1\times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C . Isotype control antibody (Green line) was mouse IgG ($1\text{ }\mu\text{g}/1\times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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