10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-Annexin A1/ANXA1 Antibody Picoband™ (monoclonal, 6B7F8)

Catalog # ABO16616

Product Information

Application	WB, IHC, FC
Primary Accession	P04083
Host	Mouse
Isotype	IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Annexin A1/ANXA1 Antibody Picoband™ (monoclonal, 6B7F8) . Tested in FCM, IHC, WB applications. This antibody reacts with Human.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	301
Other Names	Annexin A1, Annexin I, Annexin-1, Calpactin II, Calpactin-2, Chromobindin-9, Lipocortin I, Phospholipase A2 inhibitory protein, p35, Annexin Ac2-26, ANXA1, ANX1, LPC1
Calculated MW	38714
Application Details	Western blot, 0.25-0.5 □g/ml, Human Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 6B7F8
Immunogen	E.coli-derived human Annexin A1 recombinant protein (Position: A2-N346). Human Annexin A1 shares 88% and 89% amino acid (aa) sequence identity with mouse and rat Annexin A1, respectively.
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	ANXA1
Synonyms	ANX1, LPC1
Function	Plays important roles in the innate immune response as effector of glucocorticoid-mediated responses and regulator of the inflammatory process. Has anti-inflammatory activity (PubMed:8425544). Plays a role in glucocorticoid-mediated down-regulation of the early phase of the inflammatory response (By similarity). Contributes to the adaptive immune response by enhancing signaling cascades that are triggered by T-cell activation, regulates differentiation and proliferation of activated T cells (PubMed:17008549). Promotes the differentiation of T cells into Th1 cells and negatively regulates differentiation into Th2 cells (PubMed:17008549). Has no effect on unstimulated T cells (PubMed:17008549). Negatively regulates hormone exocytosis via activation of the formyl peptide receptors and reorganization of the actin cytoskeleton (PubMed:19625660). Has high affinity for Ca(2+) and can bind up to eight Ca(2+) ions (By similarity). Displays Ca(2+)-dependent binding to phospholipid membranes (PubMed:2532504, PubMed:8557678). Plays a role in the formation of phagocytic cups and phagosomes. Plays a role in phagocytosis by mediating the Ca(2+)-dependent interaction between phagosomes and the actin cytoskeleton (By similarity). In the context of antitumor immunity, interacts with FPR1 on dendritic cells allowing for tumor-associated antigens uptake and cross-presentation to T cells to mount an antitumor specific T cell response.
Cellular Location	Nucleus. Cytoplasm. Cell projection, cilium {ECO:0000250|UniProtKB:P46193}. Cell membrane. Membrane; Peripheral membrane protein. Endosome membrane {ECO:0000250|UniProtKB:P07150}; Peripheral membrane protein {ECO:0000250|UniProtKB:P07150}. Basolateral cell membrane {ECO:0000250|UniProtKB:P51662}. Apical cell membrane {ECO:0000250|UniProtKB:P10107}. Lateral cell membrane {ECO:0000250|UniProtKB:P10107}. Secreted. Secreted, extracellular space. Cell membrane; Peripheral membrane protein; Extracellular side. Secreted, extracellular exosome. Cytoplasmic vesicle, secretory vesicle lumen. Cell projection, phagocytic cup {ECO:0000250|UniProtKB:P10107}. Early endosome {ECO:0000250|UniProtKB:P19619}. Cytoplasmic vesicle membrane {ECO:0000250|UniProtKB:P19619}; Peripheral membrane protein {ECO:0000250|UniProtKB:P19619}. Note=Secreted, at least in part via exosomes and other secretory vesicles. Detected in exosomes and other extracellular vesicles (PubMed:25664854). Alternatively, the secretion is dependent on protein unfolding and facilitated by the cargo receptor TMED10; it results in the protein translocation from the cytoplasm into ERGIC (endoplasmic reticulum-Golgi intermediate compartment) followed by vesicle entry and secretion (PubMed:32272059). Detected in gelatinase granules in resting neutrophils (PubMed:10772777). Secretion is increased in response to wounding and inflammation (PubMed:25664854). Secretion is increased upon T-cell activation (PubMed:17008549). Neutrophil adhesion to endothelial cells stimulates secretion via gelatinase granules, but foreign particle phagocytosis has no effect (PubMed:10772777). Colocalizes with actin fibers at phagocytic cups (By similarity). Displays calcium-dependent binding to phospholipid membranes (PubMed:2532504, PubMed:8557678) {ECO:0000250|UniProtKB:P10107, ECO:0000269|PubMed:10772777, ECO:0000269|PubMed:17008549, ECO:0000269|PubMed:2532504, ECO:0000269|PubMed:25664854, ECO:0000269|PubMed:32272059, ECO:0000269|PubMed:8557678}

Tissue Location	Detected in resting neutrophils (PubMed:10772777). Detected in peripheral blood T cells (PubMed:17008549). Detected in extracellular vesicles in blood serum from patients with inflammatory bowel disease, but not in serum from healthy donors (PubMed:25664854) Detected in placenta (at protein level) (PubMed:2532504). Detected in liver.

Background

ANXA1, also known as lipocortin I or Annexin A1, is a protein that in humans is encoded by the ANXA1 gene. It is mapped to 9q21.13. ANXA1 belongs to a family of Ca(2+)-dependent phospholipid binding proteins which have a molecular weight of approximately 35,000 to 40,000 and are preferentially located on the cytosolic face of the plasma membrane. ANXA1 protein has an apparent relative molecular mass of 40 kDa, with phospholipase A2 inhibitory activity. Lower peptide concentrations possibly found in inflammatory situations elicit Ca(2+) transients without fully activating the mitogen-activated protein kinase pathway. This causes a specific inhibition of the transendothelial migration of neutrophils and a desensitization of neutrophils toward a chemoattractant challenge. These findings identified ANXA1 peptides as novel, endogenous FPR ligands and established a mechanistic basis of ANXA1-mediated antiinflammatory effects.

Images

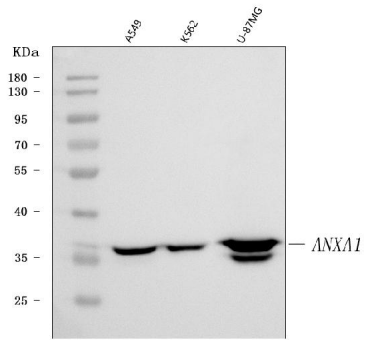


Figure 1. Western blot analysis of Annexin A1/ANXA1 using anti-Annexin A1/ANXA1 antibody (M01451-3). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human A549 whole cell lysates,
Lane 2: human K562 whole cell lysates,
Lane 3: human U-87 MG whole cell 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 A1/ANXA1 antigen affinity purified monoclonal antibody (Catalog # M01451-3) 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 Annexin A1/ANXA1 at approximately 35-39 kDa. The expected band size for Annexin A1/ANXA1 is at 39 kDa.

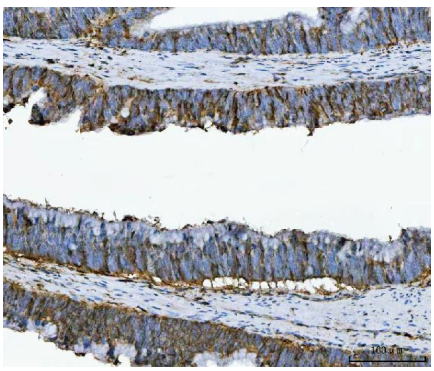


Figure 2. IHC analysis of Annexin A1/ANXA1 using anti-Annexin A1/ANXA1 antibody (M01451-3). Annexin A1/ANXA1 was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Annexin A1/ANXA1 Antibody (M01451-3) 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.

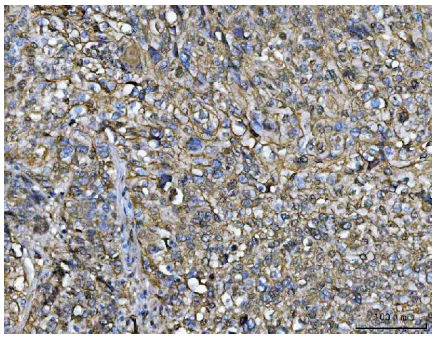


Figure 3. IHC analysis of Annexin A1/ANXA1 using anti-Annexin A1/ANXA1 antibody (M01451-3). Annexin A1/ANXA1 was detected in a paraffin-embedded section of human lung squamous cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Annexin A1/ANXA1 Antibody (M01451-3) 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.

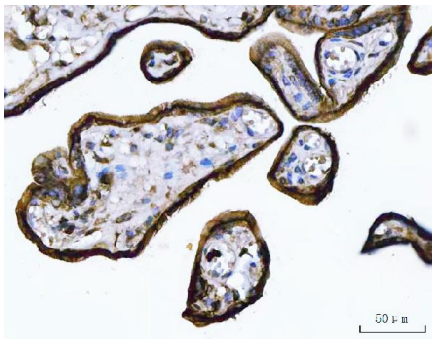


Figure 4. IHC analysis of Annexin A1/ANXA1 using anti-Annexin A1/ANXA1 antibody (M01451-3). Annexin A1/ANXA1 was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Annexin A1/ANXA1 Antibody (M01451-3) 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.

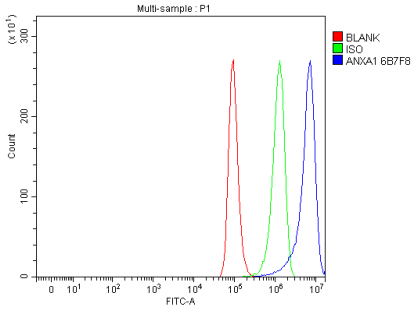


Figure 5. Flow Cytometry analysis of A549 cells using anti-Annexin A1/ANXA1 antibody (M01451-3). Overlay histogram showing A549 cells stained with M01451-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Annexin A1/ANXA1 Antibody (M01451-3, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) 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'.