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

Anti-TBP-1/PSMC3 Antibody Picoband™ (monoclonal, 4D3)

Catalog # ABO15079

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P17980
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-TBP-1/PSMC3 Antibody Picoband™ (monoclonal, 4D3) . 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 □g/ml.

Additional Information

Gene ID	5702
Other Names	26S proteasome regulatory subunit 6A, 26S proteasome AAA-ATPase subunit RPT5, Proteasome 26S subunit ATPase 3, Proteasome subunit P50, Tat-binding protein 1, TBP-1, PSMC3, TBP1
Calculated MW	49204
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 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: 4D3
Immunogen	E.coli-derived human TBP-1/PSMC3 recombinant protein (Position: M1-A439).
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	PSMC3 (HGNC:9549)
Function	Component of the 26S proteasome, a multiprotein complex involved in the ATP-dependent degradation of ubiquitinated proteins. This complex plays a key role in the maintenance of protein homeostasis by removing misfolded or damaged proteins, which could impair cellular functions, and by removing proteins whose functions are no longer required. Therefore, the proteasome participates in numerous cellular processes, including cell cycle progression, apoptosis, or DNA damage repair. PSMC3 belongs to the heterohexameric ring of AAA (ATPases associated with diverse cellular activities) proteins that unfolds ubiquitinated target proteins that are concurrently translocated into a proteolytic chamber and degraded into peptides.
Cellular Location	Cytoplasm. Nucleus. Note=Colocalizes with TRIM5 in the cytoplasmic bodies {ECO:0000250|UniProtKB:O88685}

Background

26S protease regulatory subunit 6A, also known as 26S proteasome AAA-ATPase subunit Rpt5, is an enzyme that in humans is encoded by the PSMC3 gene. The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes one of the ATPase subunits, a member of the triple-A family of ATPases that have chaperone-like activity. This subunit may compete with PSMC2 for binding to the HIV tat protein to regulate the interaction between the viral protein and the transcription complex. A pseudogene has been identified on chromosome 9.

Images

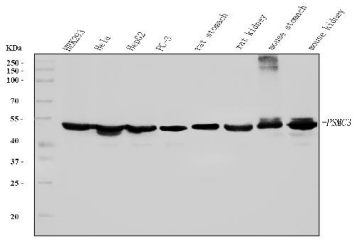


Figure 1. Western blot analysis of TBP-1/PSMC3 using anti-TBP-1/PSMC3 antibody (M07208-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human HEK293 whole cell lysates,
Lane 2: human Hela whole cell lysates,
Lane 3: human HepG2 whole cell lysates,
Lane 4: human PC-3 whole cell lysates,
Lane 5: rat stomach tissue lysates,
Lane 6: rat kidney tissue lysates,
Lane 7: mouse stomach tissue lysates,
Lane 8: mouse kidney 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-TBP-1/PSMC3 antigen affinity purified monoclonal antibody (Catalog # M07208-2) 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 TBP-1/PSMC3 at approximately 50 kDa. The expected band size for TBP-1/PSMC3 is at 50 kDa.

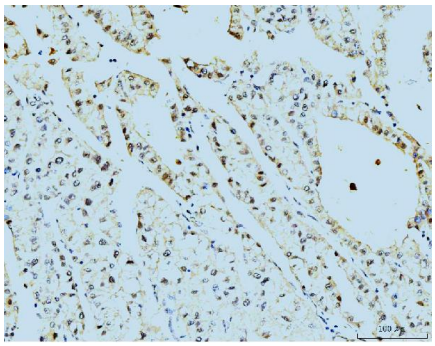


Figure 2. IHC analysis of TBP-1/PSMC3 using anti-TBP-1/PSMC3 antibody (M07208-2).
TBP-1/PSMC3 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-TBP-1/PSMC3 Antibody (M07208-2) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

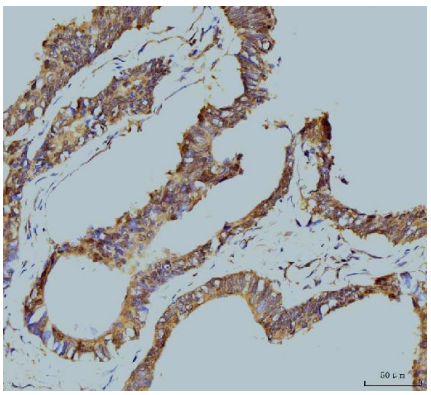


Figure 3. IHC analysis of TBP-1/PSMC3 using anti-TBP-1/PSMC3 antibody (M07208-2).
TBP-1/PSMC3 was detected in a paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-TBP-1/PSMC3 Antibody (M07208-2) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

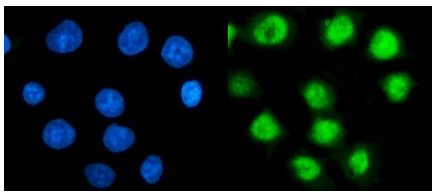
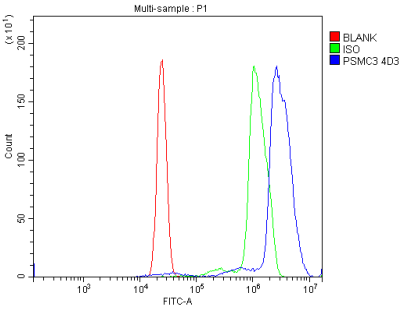


Figure 4. IF analysis of TBP-1/PSMC3 using anti-TBP-1/PSMC3 antibody (M07208-2).
TBP-1/PSMC3 was detected in an immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-TBP-1/PSMC3 Antibody (M07208-2) 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 5. Flow Cytometry analysis of U937 cells using anti-TBP-1/PSMC3 antibody (M07208-2).
Overlay histogram showing U937 cells stained with M07208-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-TBP-1/PSMC3 Antibody (M07208-2, 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'.