abcam

Product datasheet

Anti-TSG101 antibody [4A10] - BSA and Azide free ab83

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Overview

Product name	Anti-TSG101 antibody [4A10] - BSA and Azide free
Description	Mouse monoclonal [4A10] to TSG101 - BSA and Azide free
Host species	Mouse
Specificity	This antibody recognizes the TSG-101 protein, the product of a recently identified tumor susceptibility gene the inactivation of which in mouse fibroblasts results in cell transformation and the ability of those cells to form tumors in nude mice.
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment within Human TSG101 aa 150 to the C-terminus. The exact immunogen sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support team to discuss your requirements. Expressed in E.coli Database link: **Q99816**
Positive control	WB: Neuro-2a, C8D30, NIH/3T3, RAW 264.7, C2C12 , HeLa, HepG2, A431, K562 and THP-1 whole cell lysate, mouse testis. ICC/IF: Amyloid peptide-treated mouse astrocytes IHC-P: human ovarian cancer tissue, human breast carcinoma tissue. Flow cyt: THP-1 cells
General notes	This product was changed from ascites to tissue culture supernatant on 12th February 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Storage buffer	pH: 7.40 Constituent: 100% PBS w/o preservative
Carrier free	Yes
Purity	Protein G purified
Purification notes	Purified from tissue culture supernatant by Protein G chromatography to at least 95% homogeneity as determined by SDS-PAGE.
Clonality	Monoclonal
Clone number	4A10
Myeloma	NS1
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab83 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

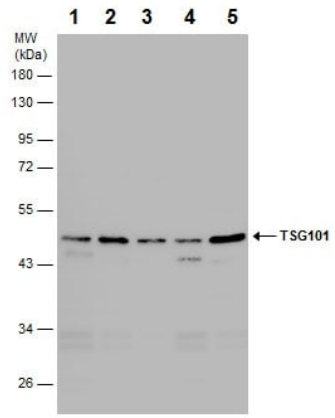
Application	Abreviews	Notes
Flow Cyt		Use at an assay dependent concentration. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ **(3)**	1/500 - 1/3000. Detects a band of approximately 47 kDa (predicted molecular weight: 43 kDa).
IHC-P		1/100 - 1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

Function	Component of the ESCRT-I complex, a regulator of vesicular trafficking process. Binds to ubiquitinated cargo proteins and is required for the sorting of endocytic ubiquitinated cargos into multivesicular bodies (MVBs). Mediates the association between the ESCRT-0 and ESCRT-I complex. Required for completion of cytokinesis; the function requires CEP55. May be involved in cell growth and differentiation. Acts as a negative growth regulator. Involved in the budding of many viruses through an interaction with viral proteins that contain a late-budding motif P-[ST]-A-P. This interaction is essential for viral particle budding of numerous retroviruses.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal, kidney and pancreas.
Sequence similarities	Belongs to the ubiquitin-conjugating enzyme family. UEV subfamily. Contains 1 SB (steadiness box) domain. Contains 1 UEV (ubiquitin E2 variant) domain.
Domain	The UEV domain is required for the interaction of the complex with ubiquitin. It also mediates the interaction with PTAP/PSAP motifs of HIV-1 P6 protein and human spumaretrovirus Gag protein.

	The coiled coil domain may interact with stathmin. The UEV domain binds ubiquitin and P-[ST]-A-P peptide motif independently.
Post-translational modifications	Monoubiquitinated at multiple sites by LRSAM1 and by MGRN1. Ubiquitination inactivates it, possibly by regulating its shuttling between an active membrane-bound protein and an inactive soluble form. Ubiquitination by MGRN1 requires the presence of UBE2D1.
Cellular localization	Cytoplasm. Membrane. Nucleus. Late endosome membrane. Mainly cytoplasmic. Membrane-associated when active and soluble when inactive. Depending on the stage of the cell cycle, detected in the nucleus. Colocalized with CEP55 in the midbody during cytokinesis.

Images



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

Lane 1 : Neuro-2a (Mouse neuroblastoma cell line) whole cell lysate
Lane 2 : C8D30 whole cell lysate
Lane 3 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
Lane 4 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 5 : C2C12 (mouse myoblast cell line) whole cell lysate

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG antibody at 1/500 dilution

Predicted band size: 43 kDa

10% SDS PAGE

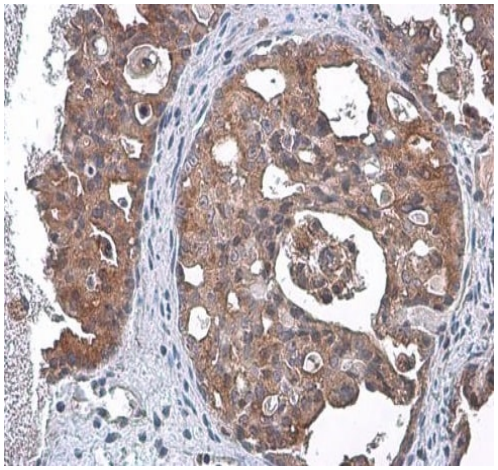
Running conditions: 80V for 15min then 140V for 40min

Blocking: 5% non-fat milk in TBST at room temperature for 60min.

Washing conditions: 5 ml TBST, 4 x 5min

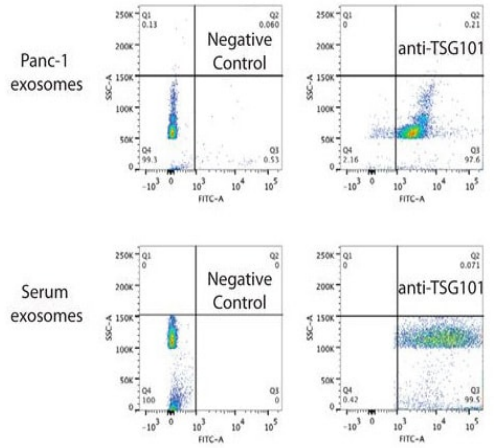
Transfer conditions: Semi-dry, 18 V, 60min (NC membrane)

Exposure system: Trident plus Western HRP Substrate



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling TSG101 with ab83 at 1/100 dilution. Cytoplasmic staining is observed. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min. (Cuisinart Electric Pressure Cooker #EPC-1200, choose "high pressure"). Endogenous peroxidase blocking: 3% H_2O_2, RT, 30min. Blocking condition: 1.5% goat serum (dilute goat serum by 1xPBS), RT, 30min. Primary antibody incubation: 4°C overnight. Secondary antibody incubation: HRP Kit (Mouse IgG), 1:200, RT, 30min. Washing: PBS, 2 x 5 minutes. DAB detection.

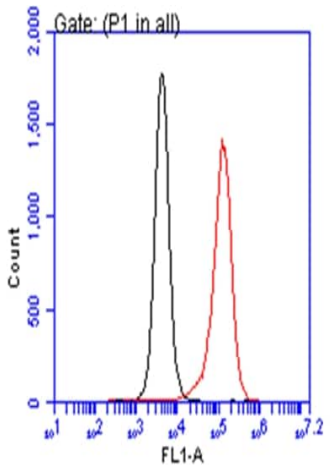


Flow Cytometry - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

Image from Kahlert C et al., J Biol Chem. 2014;289(7):3869-75. Fig 1(C).; doi: 10.1074/jbc.C113.532267.

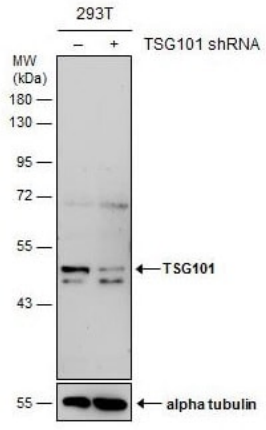
Panc-1 and Serum Exosomes were characterized by the exosome-specific expression of TSG101 by FACS analysis.

Exosomes were attached to 4-µm aldehyde/sulfate latex beads by mixing 30 µg of exosomes in a 100-µl volume of beads for 2 hours at room temperature. This suspension was diluted to 1 ml with PBS, and the reaction was stopped with 100 mm glycine and 2% BSA in PBS. Exosome-bound beads were washed in PBS/1% BSA, blocked with 10% BSA, and stained for FACS with anti-TSG101 (1:400, ab83). An Alexa Fluor® 488 conjugated anti-mouse IgG was used as the secondary antibody.



Flow Cytometry - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

Flow Cytometry analysis of THP-1 cells labeling TSG101 with ab83 at 1/25 dilution (red). Unlabelled sample was used as a control (black). A Dylight 488-conjugated secondary antibody was used for FACS analysis.



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

Lane 1 : Non-transfected (–) 293T whole cell extracts
Lane 2 : TSG101 shRNA transfected (+) 293T whole cell extracts

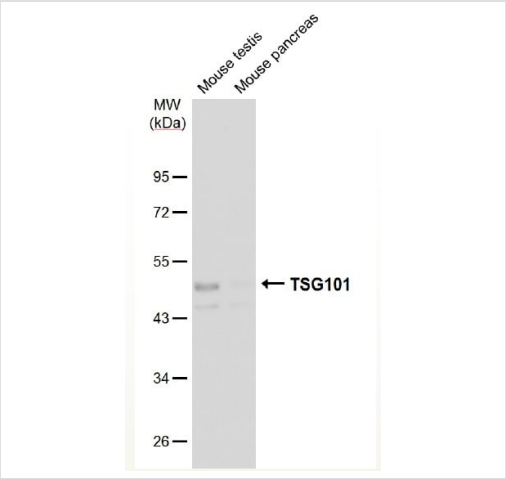
Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG antibody

Developed using the ECL technique.

Predicted band size: 43 kDa

10% SDS-PAGE



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

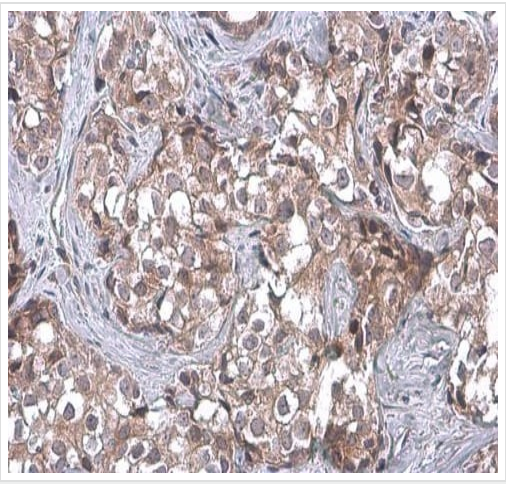
Lane 1 : Mouse testis tissue extract
Lane 2 : Mouse pancreas tissue extract

Lysates/proteins at 50 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG antibody

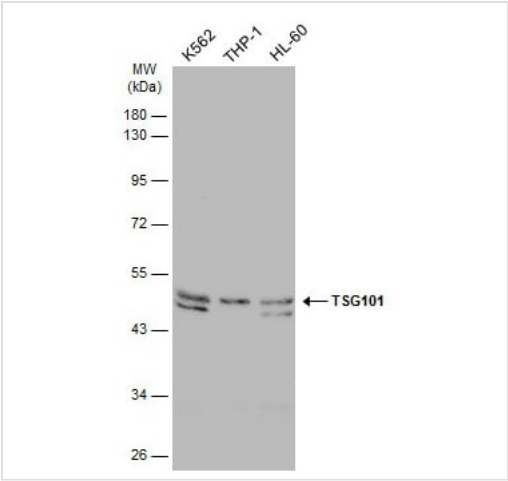
Developed using the ECL technique.

Predicted band size: 43 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling TSG101 with ab83 at 1/100 dilution. Cytoplasmic staining is observed. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

Lane 1 : K562 whole cell extract
Lane 2 : THP-1 whole cell extract
Lane 3 : HL-60 whole cell extract

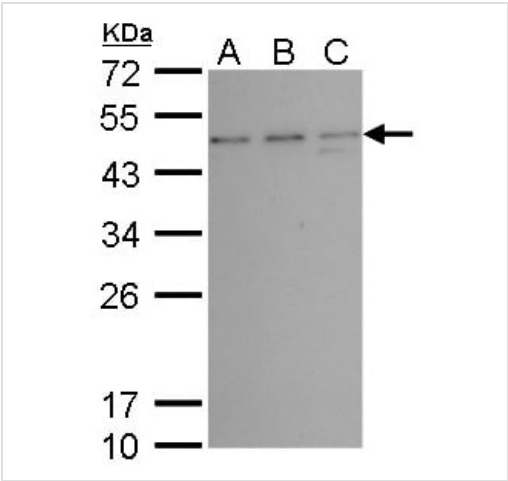
Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG antibody

Developed using the ECL technique.

Predicted band size: 43 kDa

10% SDS-PAGE



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

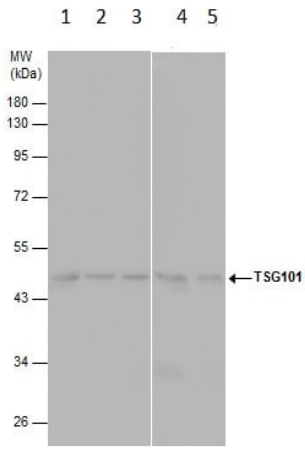
Lane 1 : NIH-3T3 whole cell lysate/extract
Lane 2 : JC (mouse mammary adenocarcinoma) whole cell lysate/extract
Lane 3 : BCL-1 whole cell lysate/extract

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP-conjugated anti-mouse IgG antibody

Predicted band size: 43 kDa

10% SDS-PAGE



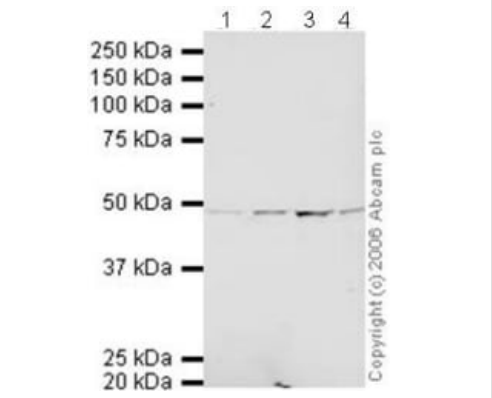
Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1/500 dilution

Lane 1 : A431 whole cell lysate
Lane 2 : HeLa whole cell lysate
Lane 3 : HepG2 whole cell lysate
Lane 4 : K562 whole cell lysate
Lane 5 : THP-1 whole cell lysate

Lysates/proteins at 30 µg per lane.

Predicted band size: 43 kDa



Western blot - Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83)

All lanes : Anti-TSG101 antibody [4A10] - BSA and Azide free (ab83) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A-431 whole cell lysate (**ab7909**)
Lane 3 : Jurkat whole cell lysate (**ab7899**)
Lane 4 : HEK-293 whole cell lysate (**ab7902**)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Donkey polyclonal to Mouse IgG (IRDyeTM 700DX) at 1/10000 dilution

Predicted band size: 43 kDa
Observed band size: 49 kDa

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