abcam

Product datasheet

Anti-TSG101 antibody ab30871

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Overview

Product name	Anti-TSG101 antibody
Description	Rabbit polyclonal to TSG101
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human TSG101 aa 350 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. Database link: **Q99816** (Peptide available as **ab30870**)
Positive control	This antibody gave a positive signal in the following whole cell lysates: HeLa (Human epithelial carcinoma cell line) A431 (Human epithelial carcinoma cell line) Jurkat (Human T cell lymphoblast-like cell line) HEK293 (Human embryonic kidney cell line) NIH 3T3 (Mouse embryonic fibroblast cell line) PC12 (Rat adrenal pheochromocytoma cell line) This antibody also gave a positive signal in human placenta tissue sections.
General notes	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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab30871 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
WB	★★★★★ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 48,49 kDa (predicted molecular weight: 44 kDa).Can be blocked with **TSG101 peptide (ab30870)**. The doublet seen in Western blot has been described in PMID:11427703 and may be due to internal initiation at Met_{10}.
ICC/IF	★★★★☆ (1)	Use a concentration of 5 µg/ml.
IHC-P	★★★★☆ (1)	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval 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.

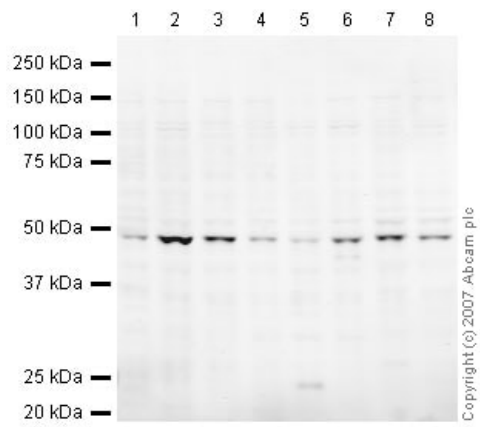
Sequence similarities Belongs to the ubiquitin-conjugating enzyme family. UEV subfamily.
Contains 1 SB (steadiness box) domain.
Contains 1 UEV (ubiquitin E2 variant) domain.

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 (ab30871)

All lanes : Anti-TSG101 antibody (ab30871) at 1 µg/ml

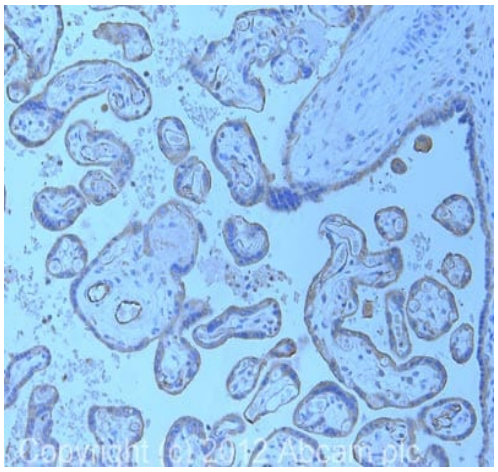
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
Lane 4 : HEK293 Human embryonic kidney cell line Whole Cell Lysate
Lane 5 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 6 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate
Lane 7 : SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate
Lane 8 : U2OS (Human osteosarcoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

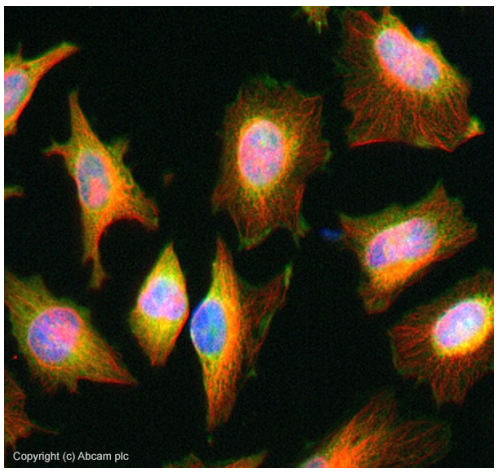
Predicted band size: 44 kDa
Observed band size: 49 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TSG101 antibody (ab30871)

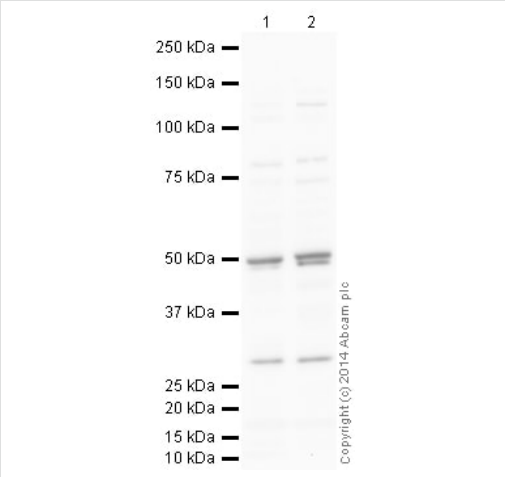
IHC image of ab30871 staining in human placenta formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab30871, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-TSG101 antibody (ab30871)

ab30871 stained in HeLa cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% Triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab30871 at 5µg/ml and **ab7291** (Mouse monoclonal to alpha Tubulin - Loading Control) used at a 1/1000 dilution overnight at +4°C. The secondary antibodies were **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed, (pseudo-colored green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594) preadsorbed, (colored red), both used at a 1/1000 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1hour at room temperature.



Western blot - Anti-TSG101 antibody (ab30871)

All lanes : Anti-TSG101 antibody (ab30871) at 1 µg/ml

Lane 1 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 2 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 44 kDa
Observed band size: 48,49 kDa
Additional bands at: 29 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 90 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab30871 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.

The doublet seen in Western blot has been described in PMID:11427703 and may be due to internal initiation at Met_{10}.

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