abcam

Product datasheet

Anti-STUB1/CHIP antibody [EPR4447] ab134064

Recombinant RabMAb

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Overview

Product name	Anti-STUB1/CHIP antibody [EPR4447]
Description	Rabbit monoclonal [EPR4447] to STUB1/CHIP
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Flow Cyt (Intra), ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human STUB1/CHIP aa 1-100 (N terminal). The exact sequence is proprietary.
Positive control	HeLa, MCF-7, 293T and ECV-304 cell lysates; Human skeletal muscle tissue.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4447
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab134064 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	★★★★☆ **(5)**	1/10000 - 1/50000. Predicted molecular weight: 35 kDa.
IP	★★★★★ **(2)**	1/120. **For unpurified, use 1/10 - 1/100.**
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF	★★★★☆ **(2)**	1/250 - 1/500.
IHC-P		1/100.

Target

Function E3 ubiquitin-protein ligase which targets misfolded chaperone substrates towards proteasomal degradation. Ubiquitinates NOS1 in concert with Hsp70 and Hsp40. Modulates the activity of several chaperone complexes, including Hsp70, Hsc70 and Hsp90. Mediates transfer of non-canonical short ubiquitin chains to HSPA8 that have no effect on HSPA8 degradation. Mediates polyubiquitination of DNA polymerase beta (POLB) at 'Lys-41', 'Lys-61' and 'Lys-81', thereby playing a role in base-excision repair: catalyzes polyubiquitination by amplifying the HUWE1/ARF-BP1-dependent monoubiquitination and leading to POLB-degradation by the proteasome. Mediates polyubiquitination of CYP3A4.

Tissue specificity Highly expressed in skeletal muscle, heart, pancreas, brain and placenta. Detected in kidney, liver and lung.

Pathway Protein modification; protein ubiquitination.

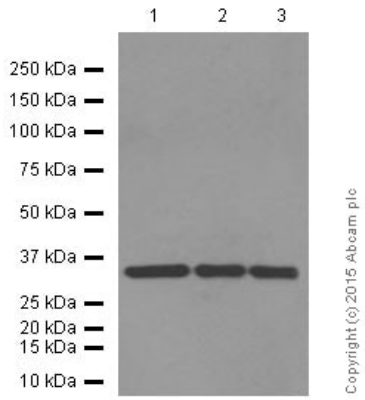
Sequence similarities Contains 3 TPR repeats.
Contains 1 U-box domain.

Domain The TPR domain is essential for ubiquitination mediated by UBE2D1.

Post-translational modifications Phosphorylated upon DNA damage, probably by ATM or ATR.
Auto-ubiquitinated; mediated by UBE2D1 and UBE2D2.

Cellular localization Cytoplasm.

Images



Western blot - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

All lanes : Anti-STUB1/CHIP antibody [EPR4447] (ab134064) at 1/20000 dilution (purified)

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) cell lysate
Lane 3 : HEK293 (Human epithelial cell line from embryonic kidney) cell lysate

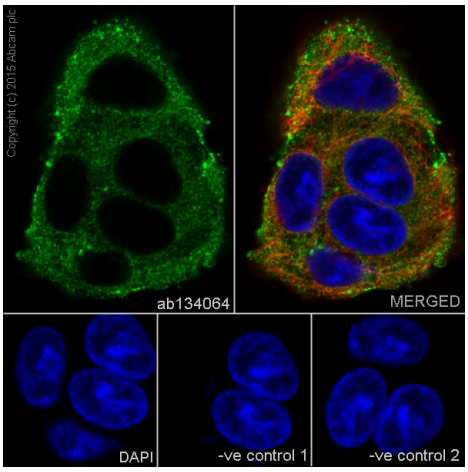
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 35 kDa
Observed band size: 35 kDa

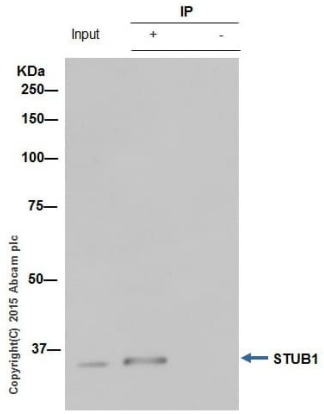
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



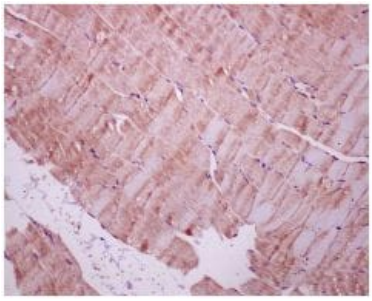
Immunocytochemistry/ Immunofluorescence - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

Immunofluorescence staining of SH-SY5Y cells with purified ab134064 at a working dilution of 1/250, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 100% methanol and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab134064 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.



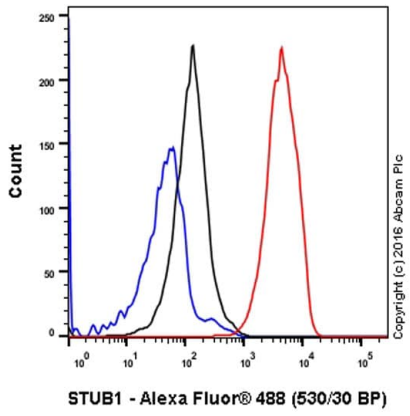
Immunoprecipitation - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

ab134064 (purified) at 1/120 immunoprecipitating STUB1/CHIP in 10 µg HeLa (Lanes 1 and 2, observed at 35 kDa). Lane 3 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



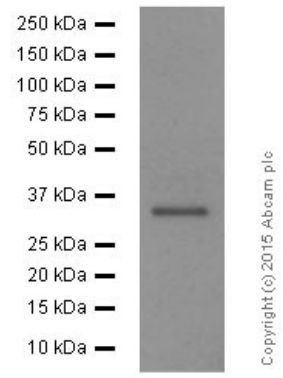
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labelling STUB1/CHIP with unpurified ab134064 at 1/100 dilution.



Flow Cytometry (Intracellular) - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

Intracellular Flow Cytometry analysis of SH-SY5Y (Human epithelial cell line from embryonic kidney) cells labeling STUB1/CHIP with purified ab134064 at 1/120 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

Anti-STUB1/CHIP antibody [EPR4447] (ab134064) at 1/10000 dilution (purified) + Mouse brain at 10 µg

Secondary

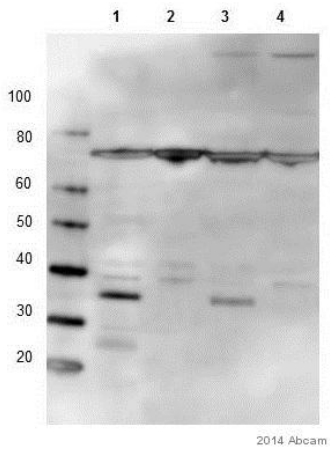
HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

Predicted band size: 35 kDa

Observed band size: 35 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

This image is courtesy of an anonymous Abreview

All lanes : Anti-STUB1/CHIP antibody [EPR4447] (ab134064) at 1/1000 dilution (unpurified)

Lane 1 : Wild type primary Mouse neurons whole cell lysate
Lane 2 : STUB1 knockout primary Mouse neurons whole cell lysate
Lane 3 : Wild type MEF (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 4 : STUB1 knockout MEF (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

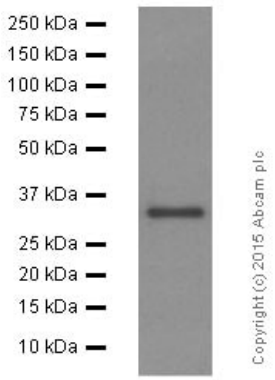
All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 35 kDa

Exposure time: 25 seconds



Western blot - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

Anti-STUB1/CHIP antibody [EPR4447] (ab134064) at 1/50000 dilution (purified) + Rat brain at 10 µg

Secondary

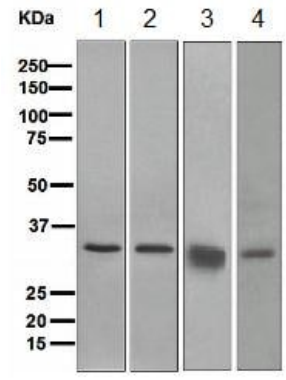
HRP goat anti-rabbit IgG (H+L) at 1/50000 dilution

Predicted band size: 35 kDa

Observed band size: 35 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

All lanes : Anti-STUB1/CHIP antibody [EPR4447] (ab134064) at 1/10000 dilution (unpurified)

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lane 2 : MCF-7 (Human breast adenocarcinoma cell line) cell lysate

Lane 3 : 293T cell lysate

Lane 4 : ECV-304 (Human urinary bladder cancer cell line) cell lysate

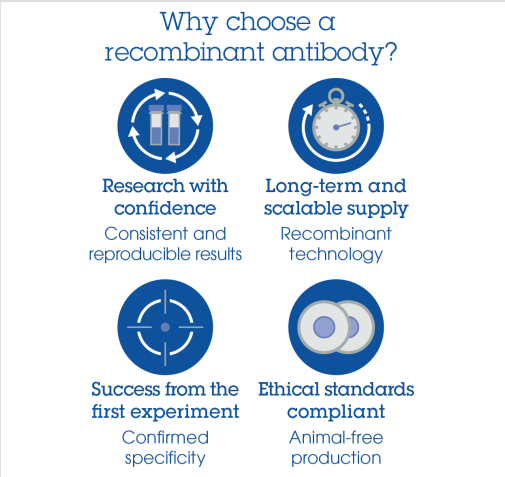
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 35 kDa

ECV-304 (derived from T24, discontinued by ATCC)



Anti-STUB1/CHIP antibody [EPR4447] (ab134064)

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