# abcam

# Product datasheet

# Anti-STUB1/CHIP antibody [EPR4447] ab134064



★★★★★ 9 Abreviews 17 References

10 Images

#### Overview

**Product name** Anti-STUB1/CHIP antibody [EPR4447]

**Description** Rabbit monoclonal [EPR4447] to STUB1/CHIP

**Host species** Rahhit

**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**.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this

update, please contact our Scientific Support team.

# **Properties**

**Form** Liquid

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Storage instructions

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	<b>★★★★</b> ★ <u>(5)</u>	1/10000 - 1/50000. Predicted molecular weight: 35 kDa.
IP	**** <u>(2)</u>	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.

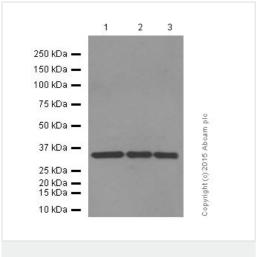
Contains 1 U-box domain.

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

**Post-translational** Phosphorylated upon DNA damage, probably by ATM or ATR. **modifications** 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.

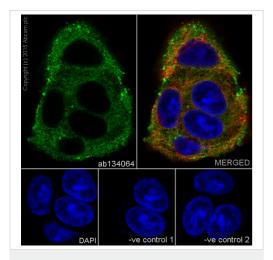
# Secondary

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

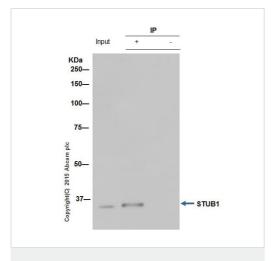
**Predicted band size:** 35 kDa **Observed band size:** 35 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

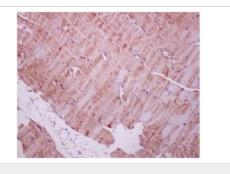


Immunocytochemistry/ Immunofluorescence - Anti-STUB1/CHIP antibody [EPR4447] (ab134064) Immunofluorescence staining of SH-SY5Y cells with purified ab134064 at a working dilution of 1/250, counter-stained with DAPI. The secondary antibody was Alexa Fluor<sup>®</sup> 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse antitubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor<sup>®</sup> 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<sup>®</sup> 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse antitubulin) was used at a dilution of 1/500 followed by an Alexa Fluor<sup>®</sup> 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.



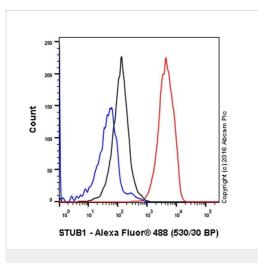
ab134064 (purified) at 1/120 immunoprecipitating STUB1/CHIP in 10  $\mu$ g HeLa (Lanes 1 and 2, observed at 35 kDa). Lane 3 - PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: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

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



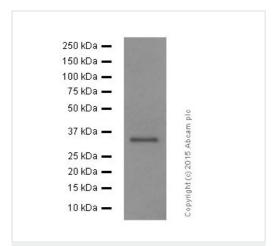
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-STUB1/CHIP antibody
[EPR4447] (ab134064)

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)

100

80 60 50 40 30 20

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

This image is courtesy of an anonymous Abreview

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

**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 lgG polyclonal at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 35 kDa

Exposure time: 25 seconds

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

# Secondary

HRP goat anti-rabbit lgG (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

1/10000 dilution (unpurified)

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

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

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

Lane 3: 293T cell lysate

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

lysate

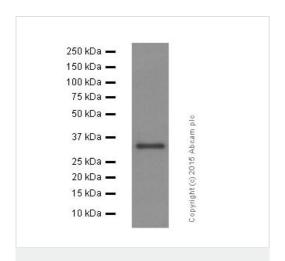
lysate

Lysates/proteins at 10 µg per lane.

# Secondary

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

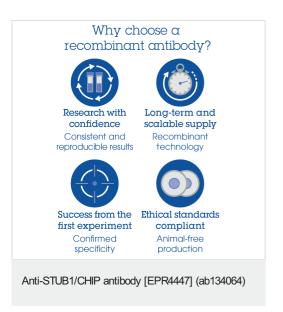
Predicted band size: 35 kDa



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



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



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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