abcam

Product datasheet

Anti-Spastin antibody [Sp 3G11/1] ab31850

敲除验证

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概述

产品名称	Anti-Spastin**抗体**[Sp 3G11/1]
描述	**小鼠单克隆抗体**[Sp 3G11/1] to Spastin
宿主	Mouse
经测试应用	**适用于:** WB, IHC-P, IP
种属反应性	**与反应:** Rat, Human
免疫原	Recombinant full length protein (Human).
阳性对照	WB: Total HeLa extract or rat brain synaptosome This antibody gave a positive result in IHC in the following FFPE tissue: Human lung adenocarcinoma. IP: HAP1 cells
常规说明	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

性能

形式	Liquid
存放说明	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.
存储溶液	Preservative: 0.02% Sodium azide Constituent: 99.98% PBS
纯度	Protein A/G purified
克隆	单克隆
克隆编号	Sp 3G11/1
骨髓瘤	Sp2/0-Ag14
同种型	IgG2a

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab31850于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

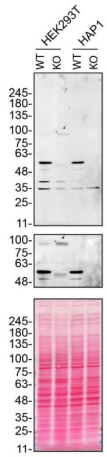
应用	Ab评论	说明
WB		1/500. Detects a band of approximately 52 kDa. There are two splice isoforms of spastin, one without exon4 and two alternative ATG start sites, which may determine the localisation of the translate protein. 52kDa is the major band.
IHC-P		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

靶标

相关性 Spastin is thought have a role in microtubule dynamics through its function as a microtubule severing protein. It is localised to the centrosome of neuronal cells but is not found in glial cells. Mutation in the ATPase binding domain of spastin causes hereditary spastic paraplegias (HSP), a large group of clinically similar disorders. Mutant forms of spastin are generally found throughout the cytoplasm rather then within the nucleus.

细胞定位 Cytoplasm, Cytoskeleton, Endoplasmic reticulum, Endosome, Membrane, Microtubule, Nucleus

图片



Western blot - Anti-Spastin antibody [Sp 3G11/1] (ab31850)

All lanes : Anti-Spastin antibody [Sp 3G11/1] (ab31850) at 1/500 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SPAST knockout HEK293T cell lysate
Lane 3 : Wild-type HAP1 cell lysate
Lane 4 : SPAST knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

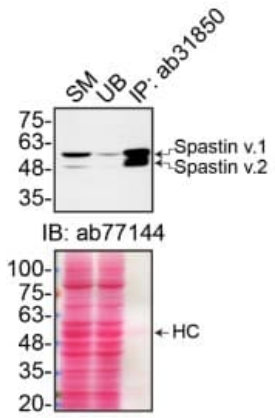
Secondary
All lanes : goat anti-rabbit HRP at 0.2 µg/ml

Performed under reducing conditions.

ab31850 was shown to react with SPAST in wild-type HEK293T cells in Western blot with loss of signal observed in SPAST knockout cell line **ab267238** (SPAST knockout cell lysate

ab258698). Wild-type HEK293T and SPAST knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab31850 overnight at 4 °C at a 1/500 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2µg/mL before imaging.

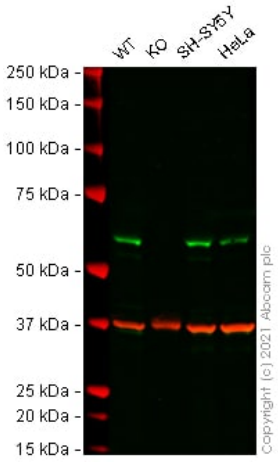
These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunoprecipitation - Anti-Spastin antibody [Sp 3G11/1] (ab31850)

Immunoprecipitation of SPAST in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1µg of ab31850 pre-coupled to Protein A beads. Samples were washed and processed for western blot with **ab77144** at 1/500.

This data was kindly provided by the YCharOS Inc., an open science company with the mission of characterizing every commercially available antibody reagent. Abcam are working with YCharOS to support their mission of antibody characterisation using knock out cell lines.



Western blot - Anti-Spastin antibody [Sp 3G11/1] (ab31850)

All lanes : Anti-Spastin antibody [Sp 3G11/1] (ab31850) at 1/500 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : Spastin knockout HEK-293T cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : HeLa cell lysate

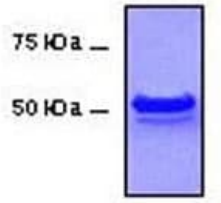
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 55 kDa

False colour image of Western blot: Anti-Spastin antibody [Sp 3G11/1] staining at 1/500 dilution, shown in green; Rabbit Anti-

GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab31850 was shown to bind specifically to Spastin. A band was observed at 55 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in SPAST knockout cell line **ab267238** (knockout cell lysate **ab258698**). To generate this image, wild-type and SPAST knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.

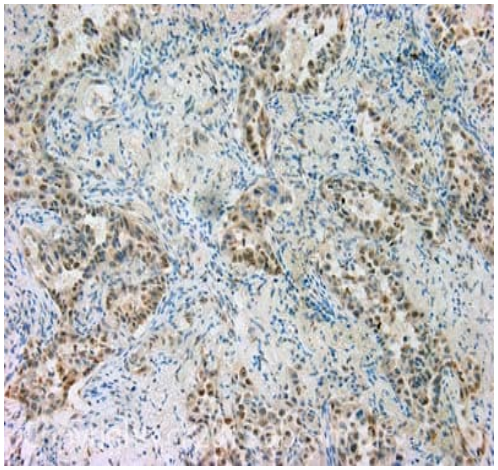


Western blot - Anti-Spastin antibody [Sp 3G11/1] (ab31850)

Anti-Spastin antibody [Sp 3G11/1] (ab31850) at 1/500 dilution + rat brain synaptosome

Observed band size: 52 kDa

There are two splice isoforms of spastin (one without exon4) and two alternative ATG start sites, which may determine the localisation of the translate protein. 52kDa is the major band.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Spastin antibody [Sp 3G11/1] (ab31850)

IHC image of Spastin staining in Human lung adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica BondTM 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 ab31850, 10µg/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.

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