abcam

Product datasheet

Anti-Spastin antibody [Sp 6C6] ab77144

KO VALIDATED

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Overview

Product name	Anti-Spastin antibody [Sp 6C6]
Description	Mouse monoclonal [Sp 6C6] to Spastin
Host species	Mouse
Tested applications	**Suitable for:** IP, WB
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Recombinant full length protein (Human)
Positive control	WB: HEK293T cell lysate; Rat brain lysate. IP: HAP1 cells
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. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	Preservative: 0.02% Sodium azide Constituent: 99.98% PBS
Purity	Protein A/G purified
Clonality	Monoclonal
Clone number	Sp 6C6
Myeloma	Sp2/0-Ag14
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab77144 in the following tested applications.

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

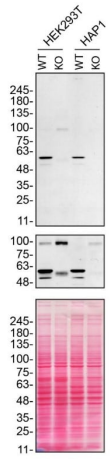
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
WB	★★★★☆ **(1)**	1/500. Predicted molecular weight: 68 kDa.

Target

Relevance 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.

Cellular localization Cytoplasm, Cytoskeleton, Endoplasmic reticulum, Endosome, Membrane, Microtubule, Nucleus

Images



Western blot - Anti-Spastin antibody [Sp 6C6] (ab77144)

All lanes : Anti-Spastin antibody [Sp 6C6] (ab77144) 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

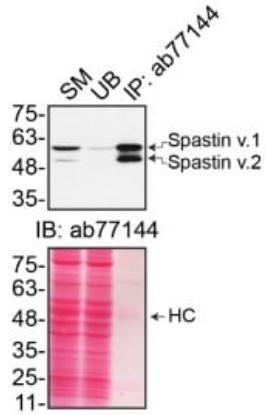
Performed under reducing conditions.

Predicted band size: 68 kDa

ab77144 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 ab77144 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.

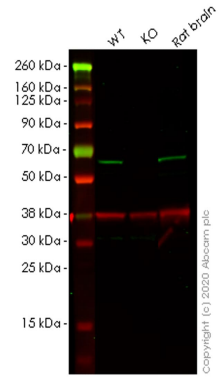
These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate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



Immunoprecipitation - Anti-Spastin antibody [Sp 6C6] (ab77144)

Immunoprecipitation of SPAST in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1µ of ab77144 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 6C6] (ab77144)

All lanes : Anti-Spastin antibody [Sp 6C6] (ab77144) at 1/500 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SPAST knockout HEK293T cell lysate
Lane 3 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

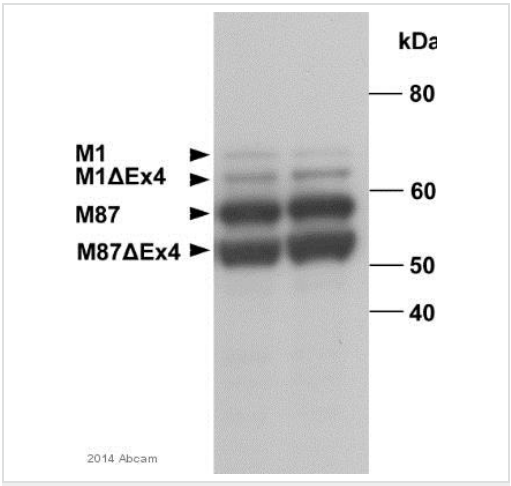
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) at 1/10000 dilution

Predicted band size: 68 kDa
Observed band size: 52 kDa

Lanes 1-3: Merged signal (red and green). Green - ab77144

observed at 52 kDa. Red - loading control **ab181602** observed at 36 kDa.

ab77144 Anti-Spastin antibody [Sp 6C6] was shown to specifically react with Spastin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab267238** (knockout cell lysate **ab258698**) was used. Wild-type and Spastin knockout samples were subjected to SDS-PAGE. ab77144 and Anti-GAPDH antibody[EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Spastin antibody [Sp 6C6] (ab77144)

This image is courtesy of an anonymous Abreview

All lanes : Anti-Spastin antibody [Sp 6C6] (ab77144) at 1/1500 dilution

All lanes : Human induced pluripotent stem cell derived neuron whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : HRP-conjugated goat anti-mouse IgG polyclonal at 1/10 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 54,58,62,68 kDa

Exposure time: 12 minutes

9% SDS-PAGE.

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