Product datasheet

Anti-beta Tubulin antibody - Loading Control ab6046

53 Abreviews  536 References  9 Images

Overview

Product name: Anti-beta Tubulin antibody - Loading Control
Description: Rabbit polyclonal to beta Tubulin - Loading Control
Host species: Rabbit
Specificity: This antibody detects a single clean band at 50kD representing beta Tubulin. This band is significantly reduced by using peptide blocking.

Tested applications: Suitable for: ICC, IHC-Fr, WB, ICC/IF, ELISA, IHC-P, IP

Species reactivity: Reacts with: Mouse, Rat, Chicken, Human, Pig, Xenopus laevis, Zebrafish, Chinese hamster

Immunogen: Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human beta Tubulin. Read Abcam's proprietary immunogen policy (Peptide available as ab20775.)

Positive control: HeLa Cell lysate; A431 Cell lysate; MCF7 Cell lysate; 293 Cell lysate; HeLa Cell lysate; A431 Cell lysate; MCF7 Cell lysate; 293 Cell lysate;

General notes

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
Note: 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

Our Abpromise guarantee covers the use of ab6046 in the following tested applications.

Our Abreviews span 50 different biological systems. You will receive an Abreport for each batch, and have access to a dedicated support team experienced in the use of our antibodies.
Function

Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.

Tissue specificity

Ubiquitously expressed with highest levels in spleen, thymus and immature brain.

Involvement in disease

Cortical dysplasia, complex, with other brain malformations 6
Skin creases, congenital symmetric circumferential, 1

Sequence similarities

Belongs to the tubulin family.

Domain

The highly acidic C-terminal region may bind cations such as calcium.

Post-translational modifications

Some glutamate residues at the C-terminus are polyglutamylated, resulting in polyglutamate chains on the gamma-carboxyl group (PubMed:26875866). Polyglutamylation plays a key role in microtubule severing by spastin (SPAST). SPAST preferentially recognizes and acts on microtubules decorated with short polyglutamate tails: severing activity by SPAST increases as the number of glutamates per tubulin rises from one to eight, but decreases beyond this glutamylation threshold (PubMed:26875866).

Some glutamate residues at the C-terminus are monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella). Both polyglutamylation and monoglycylation can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of monoglycylation is still unclear. Phosphorylated on Ser-172 by CDK1 during the cell cycle, from metaphase to telophase, but not in interphase. This phosphorylation inhibits tubulin incorporation into microtubules.

Cellular localization

Cytoplasm, cytoskeleton.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200. (see Abview)</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200. (see Abview)</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/500. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200. (see PMID: 16030258)</td>
</tr>
<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Target
Lane 1 - 8: beta Tubulin antibody - Loading Control (ab6046) at 1/500 dilution

Lane 1: HeLa Cell lysate at 20 ug
Lane 2: A431 Cell lysate at 20 ug
Lane 3: MCF7 Cell lysate at 20 ug
Lane 4: 293 Cell lysate at 20 ug with beta Tubulin peptide (ab20775) at 1 ug/ml
Lane 5: HeLa Cell lysate at 20 ug with beta Tubulin peptide (ab20775) at 1 ug/ml
Lane 6: A431 Cell lysate at 20 ug with beta Tubulin peptide (ab20775) at 1 ug/ml
Lane 7: MCF7 Cell lysate at 20 ug with beta Tubulin peptide (ab20775) at 1 ug/ml
Lane 8: 293 Cell lysate at 20 ug with beta Tubulin peptide (ab20775) at 1 ug/ml

ICC/IF image of ab6046 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6046, 1µg/ml) overnight at +4°C. The secondary antibody (green) was ab150081 Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5 min).
ab6046 staining beta Tubulin in human stomach tissue by Immunohistochemistry (frozen sections). Tissue was fixed with acetone and then blocked with 5% serum for 1 hour at 23°C followed by incubation with the primary antibody at a 1/200 dilution for 1 hour at 23°C. An undiluted HRP-conjugated goat polyclonal was used as secondary antibody.

ICC/IF image of ab6046 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6046, 5µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit IgG - H&L, pre-adsorbed (ab96899) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

Adiponectin (green) was detected using adiponectin primary antibody (ab22554; 2.5 µl/mL). Beta tubulin (red) was detected using the rabbit polyclonal (ab6046) antibody. Cells were imaged by confocal microscopy, using z-stack for adipocyte-like cells.
Beta Tubulin was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Tubulin and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min. Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab6046.


Band: 50kDa: beta Tubulin.

The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6046, 1µg/ml and ab37266, 1µg/ml) overnight at +4°C. The secondary antibodies were ab150115 Alexa Fluor® 647 goat anti-mouse IgG (H+L) used at 2µg/ml for 1h and ab175652 Alexa Fluor® 405 goat anti-rabbit IgG (H+L) used at 2µg/ml for 1h. Nuclear Green LCS1 (ab138904) was used to stain the cell nuclei (green) at a dilution of 1/500.
IHC image of beta Tubulin staining in human liver carcinoma FFPE 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 (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab6046, 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.

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Secondary

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