Overview

Product name: Anti-Ctip2 antibody [25B6]
Description: Rat monoclonal [25B6] to Ctip2
Host species: Rat
Specificity: Detects 2 bands representing Ctip2 at about 120kD. Ctip2 is highly expressed in brain and in malignant T-cell lines derived from patients with adult T-cell leukemia/lymphoma.

Tested applications:
- Suitable for: ICC/IF, WB, Flow Cyt, IHC-P
- Unsuitable for: IHC-Fr

Species reactivity: Reacts with: Mouse, Human

Immunogen:
- Recombinant fragment corresponding to Human Ctip2. GTS fusion
- Database link: Q9C0K0

Epitope: Between amino acids 1-150 of CTIP2.

Positive control:
- Flow Cyt: Jurkat cells.
- ICC/IF: Neonatal mouse hippocampal cultured neurons, Jurkat cells.
- WB: Nuclear extract from Jurkat cells; Mouse brain tissue lysate.

General notes:
- Hybridoma produced by fusion of a rat lymphocyte and mouse myeloma.

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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.
- Avoid freeze / thaw cycle.

Storage buffer:
- pH: 7.50
- Preservative: 0.02% Sodium azide
- Constituents: 0.357% HEPES, 0.87% Sodium chloride

Purity: Multi-step Chromatography
Clonality  Monoclonal
Clone number  25B6
Isotype  IgG2a

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab18465 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐ ⚫ (10)</td>
<td>1/500.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ (3)</td>
<td>Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 95 kDa).</td>
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<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐ (1)</td>
<td>Use 1µg for 10⁶ cells. ab18450 - Rat monoclonal IgG2a, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐ (10)</td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
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Application notes Is unsuitable for IHC-Fr.

Target

Function  Tumor-suppressor protein involved in T-cell lymphomas. May function on the P53-signaling pathway. May be a key regulator of both differentiation and survival during thymocyte development. Repress transcription through direct, TFCOUP2-independent binding to a GC-rich response element.

Tissue specificity  Highly expressed in brain and in malignant T-cell lines derived from patients with adult T-cell leukemia/lymphoma.

Sequence similarities  Contains 6 C2H2-type zinc fingers.

Cellular localization  Nucleus.

Images
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ctip2 antibody [25B6] (ab18465)

Negative control image: IHC image of Ctip2 staining in a section of formalin-fixed paraffin-embedded mouse cerebellum performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab18465, 5ug/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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ctip2 antibody [25B6] (ab18465)

IHC image of Ctip2 staining in a section of formalin-fixed paraffin-embedded mouse hippocampus performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab18465, 5ug/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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ctip2 antibody [25B6] (ab18465)

Negative control image: IHC image of Ctip2 staining in a section of formalin-fixed paraffin-embedded human cerebellum* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab18465, 5ug/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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ctip2 antibody [25B6] (ab18465)

IHC image of Ctip2 staining in a section of formalin-fixed paraffin-embedded human hippocampus* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab18465, 5ug/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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
Immunofluorescence staining of Ctip2 using ab18465 in Jurkat cells (+ve expression control, top panel) and Daudi cells (-ve expression control, bottom panel). The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton-X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab18465 at 0.2 µg/mL and ab6046 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed (ab150165) (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (ab150084) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Immunofluorescence staining of Ctip2 using ab18465 in Jurkat cells (+ve expression control, top panel) and Daudi cells (-ve expression control, bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton-X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab18465 at 0.2 µg/mL and ab6046 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed (ab150165) (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (ab150084) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).
Neonatal mouse hippocampal neurons stained with ab18465 (top panel - green) and Ctip1 antibody (middle - red). Bottom panel is overlay of ab18465 and Ctip1 antibody staining - yellow indicates co-localisation, green is ab18465 alone and red is Ctip1 antibody alone.

Western blot using ab18465 on nuclear extract from Jurkat cells immunoprecipitated with anti-Sir2 antibody.

Two bands are seen which may correspond to two CTIP2 transcripts present in Jurkat cells as previously reported (Bernard et al. 2001).

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Neonatal Mouse Hippocampal Neurons (Harvested at P1, grown 5d in culture on glial cell feeder layer).

Red is beta tubulin staining.
Green is ab18465.
Overlay histogram showing Jurkat cells stained with ab18465 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab18465, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rat IgG (H+L) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rat IgG (2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.

All lanes : Anti-Ctip2 antibody [25B6] (ab18465) at 1/500 dilution

Lanes 1-2 : Mouse brain tissue lysate at 1.5 µg
Lane 3 : Mouse brain tissue lysate at 3 µg

Secondary
All lanes : IRDYE 680-conjugated Donkey Anti-Rat polyclonal. at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 95 kDa
Observed band size: 100,110 kDa

Exposure time: 10 minutes

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

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