# Product datasheet

## Anti-Iba1 antibody ab5076

<table>
<thead>
<tr>
<th><strong>Overview</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Product name</strong></td>
<td>Anti-Iba1 antibody</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Goat polyclonal to Iba1</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Goat</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>This antibody, ab5076, is expected to recognise the isoforms represented by NP_001614 and NP_116573 but not NP_004838. Please note: Although we have some very good Abreviews on mouse, some customers were receiving inconsistent results on mouse samples. We have therefore moved mouse to the predicted species and can no longer guarantee it. In addition, we recommend blocking in milk. Blocking with BSA gives high background. IHC-Fr. Some customers have used this product in IHC-Fr however we have had consistent complaints in this application over the last year and can no longer guarantee performance in this application. If looking for a monoclonal anti-Iba1 alternative we can recommend our RabMAb ab178846. This has the same immunogen as ab5076.</td>
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| **Tested applications** | Suitable for: Electron Microscopy, IHC-Fr, IHC-P, WB, IHC-FrFl, ICC, ICC/IF  |
| **Unsuitable for:** | IHC-FoFr |

| **Species reactivity** | Reacts with: Rabbit, Guinea pig, Cow, Dog, Human, Pig, Common marmoset  |
| **Predicted to work with:** | Mouse, Rat, Macaque monkey |

| **Immunogen** | Synthetic peptide corresponding to Human Iba1 aa 135-147 (C terminal). Accession Number(s): NP_116573.1; NP_001614.3  |
| **Sequence:** | C-TGPPAKKAISELP  |
| **(Peptide available as ab23067)** |  |

| **Positive control** | Recombinant Human Iba1 protein (ab117478) can be used as a positive control in WB.  |
| **General notes** | Please note: Although we have some very good Abreviews on mouse, some customers were receiving inconsistent results on mouse samples. We have therefore moved mouse to the predicted species and can no longer guarantee it.  |

| **Properties** |  |
| **Form** | Liquid |
Storage instructions
Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer
pH: 7.3
Preservative: 0.02% Sodium azide
Constituents: Tris buffered saline, 0.5% BSA

Purity
Immunogen affinity purified

Purification notes
Purified from goat serum by ammonium sulphate precipitation followed by antigen affinity chromatography using the immunizing peptide.

Clonality
Polyclonal

Isotype
IgG

Applications
Our Abpromise guarantee covers the use of ab5076 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Microscopy</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 26358247</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>1/500.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 2 - 4 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 0.3 - 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa). Can be blocked with Human Iba1 peptide (ab23067). We received several excellent Abreviews on this antibody working with mouse, however, mouse is not a batch tested species and we cannot guarantee that this antibody will work on mouse. Some of our customers have observed high background in mouse samples. We recommend blocking in milk. Blocking with BSA gives high background.</td>
</tr>
<tr>
<td>IHC-FrFl</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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</table>

Application notes
Is unsuitable for IHC-FoFr.

Target

Function

Tissue specificity
Detected in T-lymphocytes and peripheral blood mononuclear cells.

Sequence similarities
Contains 2 EF-hand domains.
Post-translational modifications

Phosphorylated on serine residues.

Cellular localization

Cytoplasm > cytoskeleton. Cell projection > ruffle membrane. Associated with the actin cytoskeleton at membrane ruffles and at sites of phagocytosis.

Images

Paraffin embedded sections of human lung were stained for Iba1 with ab5076 at 5 μg/ml in immunohistochemical analysis.

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab5076)**

**Western blot - Anti-Iba1 antibody (ab5076)**

All lanes: Anti-Iba1 antibody (ab5076) at 0.3 μg/ml

Lane 1: Rat Brain cell lysate (35μg protein in RIPA buffer)

Lane 2: Human Frontal Cortex cell lysate (35μg protein in RIPA buffer)

Predicted band size: 17 kDa

Primary incubation was 1 hour. Detected by chemiluminescence.

Free floating sections of rat striatum/microglia were stained for Iba1 with ab5076 at 1/2000 dilution in immunohistochemical analysis.

Rabbit Anti-Goat IgG Biotin was used as the secondary antibody at 1/200 dilution.
Paraffin embedded sections of mouse brain were stained for Iba1 with ab5076 at 2 µg/ml in immunohistochemical analysis.

IHC-P image of Iba1 staining on rat brain sections using ab5076 (1:1000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab5076 was diluted 1:1000 and incubted with the sections for 2 hours at 21°C. The secondary antibody used was Rabbit polyclonal to anti sheep/goat IgG conjugated to biotin (1:200)

All lanes: Anti-Iba1 antibody (ab5076) at 1 µg/ml

Lane 1: Human Iba1 full length recombinant protein at 0.1 µg
Lane 2: HEK293 whole cell lysate at 30 µg
Lane 3: A431 whole cell lysate at 30 µg
Lane 4: NIH3T3 whole cell lysate at 30 µg
Lane 5: Human spleen tissue lysate at 30 µg
Lanes 6 & 15: Mouse spleen tissue lysate at 30 µg
Lane 7: Rat spleen tissue lysate at 30 µg
Lane 8: U937 whole cell lysate at 30 µg
Lane 9: HL60 whole cell lysate at 30 µg
Lanes 10 & 16: THP1 whole cell lysate at 30 µg
Lane 11: THP1 whole cell lysate, PMA treated at 30 µg
Lane 12: Raw 264.7 whole cell lysate at 30 µg
Lane 13: C6 whole cell lysate at 30 µg
Lane 14: NR8383 whole cell lysate at 30 µg
Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 17 kDa

Lanes 1-14: Blocked in 3% milk for 1 hour (RT). Lanes 15-16: Blocked in 5% BSA for 1 hour (RT).

Lane 1: exposure 1 minute. Lanes 2-16: exposure 4 minutes.

Abcam recommends blocking in milk for cleaner blots with reduced background, in comparison to BSA.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes.

The membrane was then blocked for an hour before being incubated with ab5076 (anti-Iba1 antibody; 1 ug/mL) for 18 hours at 4°C.

Frozen sections of mouse brain tissue were stained for Iba1 with ab5076 at 1/500 dilution in immunohistochemical analysis. Rabbit Anti-Goat IgG HRP was used as the secondary antibody at 1/500 dilution.
ab5076 staining Iba1 in the Macrophage cell line Mono-Mac 6 by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 10% serum for 20 minutes at 24°C. Samples were incubated with primary antibody (1/500 in PBS) for 16 hours at 4°C. An Alexa Fluor® 555-conjugated Rabbit anti-goat IgG polyclonal (1/1000) was used as the secondary antibody.

IHC-P image of Iba1 staining on cat kidney sections using ab5076 (1:1000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab5076 was diluted 1:1000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was Rabbit polyclonal to anti sheep/goat IgG conjugated to biotin (1:200).
Anti-Iba1 antibody (ab5076) at 0.3 µg/ml + Rat brain lysate at 35 µg

**Predicted band size:** 17 kDa

**Observed band size:** 17 kDa

Primary incubation was 1 hour. Detected by chemiluminescence.

ab5076 staining Iba1 in rat glioblastoma cell line C6 by Immunocytochemistry/Immunofluorescence. Cells were fixed in paraformaldehyde, permeabilized using 0.1% Triton X 100 in PBS, blocked with 0.5% BSA for 30 minutes at room temperature and then incubated with ab5076 at a 1/50 dilution for 16 hours at 4°C. The secondary used was a Cy3 conjugated rabbit anti-goat polyclonal used at a 1/120 dilution. Nuclei are counterstained with DAPI.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab5076)

This image is courtesy of an abreview submitted by Carl Hobbs (King's College London, United Kingdom)

IHC-P image of Iba1 staining on cow kidney sections using ab5076 (1:2000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab5076 was diluted 1:2000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was Rabbit polyclonal to anti sheep/goat IgG conjugated to biotin (1:200)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Iba1 antibody (ab5076)

This image is courtesy of an abreview submitted by Carl Hobbs (King's College London, United Kingdom)

IHC-P image of Iba1 staining on marmoset bladder sections using ab5076 (1:1000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab5076 was diluted 1:1000 and incubated with the sections for 2 hours at 21°C. The secondary antibody used was Rabbit polyclonal to anti goat IgG conjugated to biotin (1:200)

AIF1 expression in an inflammatory foci in a skin biopsy. Paraffin embedded sections were deparaffinized and boiled in 10mM citrate buffer for 30 min in a microwave to expose the AIF1 antigen, and then blocked with 5% donkey serum for 20 min. Slides were incubated for 40 min with goat-anti-AIF1 antibody (1:100) and rinsed in three changes of PBS for 1 min each. A secondary antibody (donkey-anti-goat-FITC conjugated IgG; 1:50) was then applied to the slide for 40 min. Sections were washed again to remove the unbound antibody. The slides were counterstained with DaPI and viewed with a Nikon epi-fluorescent microscope. AIF1 positive cells appear green and the nuclei are stained blue. Numerous cells expressing the AIF1 protein are located around a vessel. (400X). Picture from Rreview by Carol Artlett submitted 30 July 2004.
ab5076 staining Iba1 in guinea pig brain tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent formaldehyde fixation before heat mediated antigen retrieval in sodium citrate and then blocking with 3% serum was performed for 30 minutes at RT. The primary antibody was used at dilution at 1/200 and incubated with sample at 2% blocking serum for 18 hours at 4°C. A Biotin conjugated horse polyclonal to goat IgG was used undiluted as secondary antibody.

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