Product datasheet

Anti-Mast Cell Tryptase antibody [AA1] ab2378

Overview

Product name
Anti-Mast Cell Tryptase antibody [AA1]

Description
Mouse monoclonal [AA1] to Mast Cell Tryptase

Host species
Mouse

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

Species reactivity
Reacts with: Mouse, Rat, Human

Predicted to work with: Cat, Dog, Monkey

Immunogen
Full length native protein (purified) corresponding to Human Mast Cell Tryptase. Human mast cell tryptase was purified from lung tissue by high salt extraction, ammonium sulphate precipitation, octyl Sepharose and heparin-agarose chromatography. (PMID 2253091)

Positive control
WB: Human lung, tonsil and skin tissue lysate. IHC-P: Human skin tissue.

General notes
This antibody clone is manufactured by Abcam.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

Properties

Form
Liquid

Storage instructions
Shipped at 4°C. Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.40
Preservative: 0.02% Sodium azide

Purity
Tissue culture supernatant

Clonality
Monoclonal

Clone number
AA1

Myeloma
unknown

Isotype
IgG1

Light chain type
unknown

Applications
Our Abpromise guarantee covers the use of ab2378 in the following tested applications.

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

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<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ICC</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐ 1/100 - 1/500. Detects a band of approximately 35-37 kDa (predicted molecular weight: 31 kDa). We recommend using 3% milk as the blocking agent for Western blot.</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐ 1/200000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
<td></td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐ 1/100.</td>
<td></td>
</tr>
<tr>
<td>IHC-R</td>
<td>⭐⭐⭐⭐⭐ Use at an assay dependent concentration.</td>
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<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐ Use at an assay dependent concentration.</td>
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</tbody>
</table>

**Target**

**Relevance**

Mast cells contain a number of preformed chemical mediators such as histamine, chymase, carboxypeptidase and proteolytic tryptase. Human Mast Cell Tryptase is considered to be an important marker of mast cell activation as well as an important mediator of inflammation.

**Cellular localization**

Secreted. Note: released from the secretory granules upon mast cell activation.

**Images**

IHC image of Mast Cell Tryptase staining in Human Normal Skin 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) for 20 mins. The section was then incubated with ab2378, 1/200,000 dilution, 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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*
Lanes 1-3: Anti-Mast Cell Tryptase antibody [AA1] (ab2378) at 1/100 dilution
Lanes 4-6: Anti-Mast Cell Tryptase antibody [AA1] (ab2378) at 1/500 dilution

Lanes 1 & 4: Human lung normal tissue lysate - total protein (40 - 65 years)
Lanes 2 & 5: Human tonsil normal tissue lysate - total protein
Lanes 3 & 6: Human skin tissue lysate - total protein

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Mouse IgG H&L (HRP) preadsorbed at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 31 kDa

Exposure time: 2 minutes

Mast Cell Tryptase contains an 18-residue signal sequence and a 12-residue activation peptide. The protein also has two potential glycosylation sites (Swissprot data). These post-translational modifications might explain the banding pattern observed. Abcam used 5% milk in TBS-T as a blocking agent for this blot. We found that this blocking agent removed more non-specific bands than BSA.
**Tryptase-stained skin biopsy.**

Skin biopsy in CUrt patient stained for tryptase (ab2378, red) at baseline at low (10×, upper panels) and high (60×, lower panels) magnification and at 15 minutes following cold stimulation time test.

Skin biopsies were obtained at baseline and 15 minutes after CSTT (5 min) on the challenge site, placed into 10% neutral buffered formalin, embedded in paraffin and cut into 5-µm-thick sections. Local anesthesia was performed just before biopsy and did not delay the timing of the biopsy. Slides for immunohistochemistry were deparaffinized and stained. ab2378 was diluted 1:100 and incubated for 2 hours. A biotinylated undiluted secondary antibody of Goat anti-Mouse SS Link, was incubated for 32 minutes, followed with enzyme conjugate, and Fast Red chromogen.

ab2378 staining formalin fixed paraffin-embedded human colon tissue sections cut at 1 micron.

The section was subjected to heat mediated antigen retrieval and permeabilized in Triton-X prior to blocking in 6% BSA for 2 hours at 24°C. The primary antibody was diluted 1/250 and incubated with the sample for 16 hours at 5°C. The secondary antibody was ab7064 (diluted 1/2000) and the slide was counterstained with DAPI.

**AM distribution in thymic mast cells in cryostat sections of human newborn thymic tissue.**

Sections were incubated with an antibody against tryptase (A, B) using ab2378 at a 1/100 dilution.

V: blood vessel.

Briefly, frozen sections (10 µm-thick) were cut with a cryostat at −20°C. Antigens were retrieved by heating the sections previously immersed in Bond Epitope Retrieval solution 1 at 100°C for 30 min. Sections were washed in phosphate buffered saline (PBS) and incubated with the primary antibody.
ab2378 staining Mast Cell Tryptase in rat Lung tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. A Biotin-conjugated Goat anti-mouse IgG polyclonal (1/500) was used as the secondary antibody.

ab2378 staining Mast Cell Tryptase in human tonsil tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in a citric acid. Samples were incubated with primary antibody (1/7500 in TBS/BSA/azide) for 2 hours at 21°C. A Biotin-conjugated Goat anti-mouse IgG polyclonal (1/250) was used as the secondary antibody.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Mast Cell Tryptase antibody [AA1] (ab2378)

Image courtesy of an AbReview submitted by Dr Francois Daubeuf

ab2378 staining Mast Cell Tryptase in mouse Lung (LPS inflammation) tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with paraformaldehyde and blocked with 4% M.O.M Ig blocking for 1 hour at 20°C; antigen retrieval was by heat mediation in a citric acid. Samples were incubated with primary antibody (1/200 in blocking buffer) for 16 hours at 4°C. A Biotin-conjugated Rabbit anti-mouse IgG polyclonal (1/100) was used as the secondary antibody.

Immunohistochemistry (Resin sections) - Anti-Mast Cell Tryptase antibody [AA1] (ab2378)

This image is courtesy of an anonymous Abreview

ab2378 staining Mast Cell Tryptase in human nasal polyp and tonsil tissue sections by Immunohistochemistry (resin-embedded sections). Tissue was fixed with acetone and inhibitors and embedded in GMA resin. Samples were incubated with primary antibody (1/2000 in Tris buffered saline) for 18 hours at 21°C. A Biotin-conjugated Rabbit anti-mouse IgG polyclonal (1/1000) was used as the secondary antibody.

Immunocytochemistry/ Immunofluorescence - Anti-Mast Cell Tryptase antibody [AA1] (ab2378)

This image is courtesy of an anonymous Abreview

ab2378 staining Mast Cell Tryptase in human mast cells by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with methanol, permeabilized with Saponin 0.1% in PBS and blocked with 4% serum for 30 minutes at 25°C. Samples were incubated with primary antibody (1/100 in blocking buffer) for 14 hours at 4°C. A Texas Red® conjugated Goat anti-mouse polyclonal (1/100) was used as the secondary antibody.

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