Product name: Anti-Cathepsin L + V antibody [33/2] ab6314

Description: Mouse monoclonal [33/2] to Cathepsin L + V

Host species: Mouse

Specificity: Reacts specifically with the native and denatured forms of human cathepsin L (25kD) and procathepsin L (42 kD). Does not react with human cathepsin types B1, D1, H and S1, procathepsins B and D1 and recombinant procathepsin H, rat cathepsin B1, and mouse procathepsins B1 and D1.

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

Species reactivity: Reacts with: Mouse, Rat, Human

Immunogen: Full length native protein (purified) corresponding to Human Cathepsin L + V.

Epitope: Recognizes an epitope within amino acid residues GYGFEST (265-271 in procathepsin L and 169-175 in the mature cathepsin L molecule).

Positive control: This antibody gave a positive signal in human and mouse lung tissue lysates and in human, mouse and rat kidney tissue lysates. In Flow Cytometry, this antibody gave a positive result in methanol fixed/Tween permeabilised HeLa cells. IHC-P: FFPE human colon adenocarcinoma tissue sections.

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.

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

Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.
Purity  
Immunogen affinity purified

Clonality  
Monoclonal

Clone number  
33/2

Isotype  
IgG1

Applications

Our Abpromise guarantee covers the use of ab6314 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>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. Detects a band of approximately 25 kDa (predicted molecular weight: 38 kDa). Abcam recommends using 3% milk as the blocking agent.</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>1/50.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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</tbody>
</table>
| Flow Cyt    |           | Use 1µg for 10^6 cells.  
ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |

Images

Lane 1 : Anti-Cathepsin L/V/K/H antibody [EPR8011] (ab133641) at 1/20 dilution
Lane 2 : Anti-Cathepsin L + V antibody [33/2] (ab6314) at 1/100 dilution
Lane 3 : Anti-His tag at 1/10000 dilution

All lanes : Human Cathepsin L recombinant protein fraction

Secondary

Lanes 1 & 3 : Peroxidase conjugated Goat Anti-Rabbit IgG, (H+L) at 1/1000 dilution
Lane 2 : Rabbit Anti-Mouse secondary ab at 1/2000 dilution

Predicted band size: 38 kDa
Blocking buffer and concentration: 5% NFDM/TBST
Diluting buffer and concentration: 5% NFDM/TBST
Observed MW: 30
Exposure time: 3 minutes

**Western blot - Anti-Cathepsin L + V antibody [33/2]**

Lane 1: Anti-Cathepsin L/V/K/H antibody [EPR8011] (ab133641) at 1/20 dilution
Lane 2: Anti-Cathepsin L/V/K/H antibody [EPR8011] (ab133641) at 1/100 dilution
Lane 3: Anti-Cathepsin L/V/K/H antibody [EPR8011] (ab133641) at 1/500 dilution
Lane 4: Anti-Cathepsin L + V antibody [33/2] (ab6314) at 1/100 dilution
Lane 5: Anti-Cathepsin L + V antibody [33/2] (ab6314) at 1/1000 dilution
Lane 6: Anti-His tag at 1/10000 dilution

All lanes: Human Cathepsin V recombinant protein fraction

**Secondary**
Lanes 1-3: Peroxidase conjugated Goat Anti-Rabbit IgG, (H+L) at 1/1000 dilution
Lanes 4-5: Rabbit Anti-Mouse secondary ab at 1/2000 dilution
Lane 6: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 38 kDa
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cathepsin L + V antibody [33/2] (ab6314)

IHC image of Cathepsin L staining in human colon adenocarcinoma formalin fixed paraffin embedded tissue 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 (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab6314, 0.5µg/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.

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

Western blot - Anti-Cathepsin L + V antibody [33/2] (ab6314)

All lanes: Anti-Cathepsin L + V antibody [33/2] (ab6314) at 1/1000 dilution (3% milk block)

Lane 1: Lung (Human) Tissue Lysate
Lane 2: Lung (Mouse) Tissue Lysate
Lane 3: Human kidney tissue lysate - total protein (ab30203)
Lane 4: Kidney (Mouse) Tissue Lysate
Lane 5: Kidney (Rat) Tissue Lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 38 kDa
Observed band size: 25 kDa

why is the actual band size different from the predicted?

Additional bands at: 55 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 20 minutes
Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.

Anti-Cathepsin L + V antibody [33/2] (ab6314) at 1/750 dilution + whole cell lysate prepared from U87MG human glioblastoma at 50 µg

**Secondary**
Goat anti-mouse HRP at 1/2000 dilution

**Predicted band size:** 38 kDa

ab6314 at a 1/150 dilution staining Cathepsin L in CHO cells by Immunocytochemistry/Immunofluorescence.
Overlay histogram showing HeLa cells stained with ab6314 (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 (ab6314, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

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

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