

Anti-Ubiquitin antibody [Ubi-1] ab7254

★★★★★ 6 Abreviews 86 References 5 Images

Overview

| | |
|----------------------------|---|
| Product name | Anti-Ubiquitin antibody [Ubi-1] |
| Description | Mouse monoclonal [Ubi-1] to Ubiquitin |
| Host species | Mouse |
| Tested applications | Suitable for: IHC-P, WB Unsuitable for: IP |
| Species reactivity | Reacts with: Mouse, Human |
| Immunogen | Full length protein corresponding to Cow Ubiquitin conjugated to keyhole limpet haemocyanin (Glutaraldehyde). |
| Positive control | H9C2 cells IHC-P:FFPE mouse brain alzheimer |
| General notes | <p>This product was changed from ascites to tissue culture supernatant on 17th May 2016. The following lots are from ascites and are still in stock as of 17th May 2016: GR159581-2, GR159581-12, GR159581-13. Lot numbers higher than GR159581-13 will be from tissue culture supernatant. Please note that the dilutions may need to be adjusted accordingly.</p> <p>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.</p> <p>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</p> |

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 long term. Avoid freeze / thaw cycle. |
| Storage buffer | Preservative: 0.1% Sodium azide |
| Purity | Affinity purified |
| Clonality | Monoclonal |
| Clone number | Ubi-1 |

Isotype IgG1
Light chain type kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab7254 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|-------------|-----------|--|
| IHC-P | | Use a concentration of 1 - 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| WB | ★★★★★ (4) | 1/100 - 1/5000. |

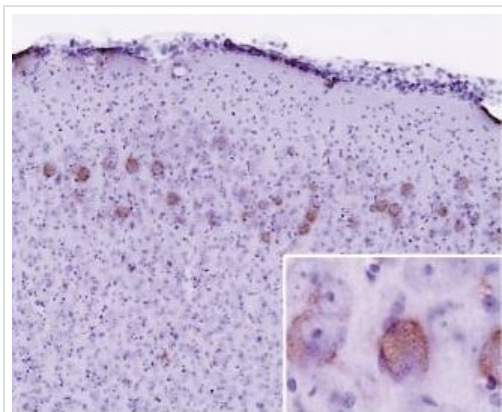
Application notes Is unsuitable for IP.

Target

Relevance Function: Ubiquitin exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). Polyubiquitin chains, when attached to a target protein, have different functions depending on the Lys residue of the ubiquitin that is linked: Lys-6-linked may be involved in DNA repair; Lys-11-linked is involved in ERAD (endoplasmic reticulum-associated degradation) and in cell-cycle regulation; Lys-29-linked is involved in lysosomal degradation; Lys-33-linked is involved in kinase modification; Lys-48-linked is involved in protein degradation via the proteasome; Lys-63-linked is involved in endocytosis, DNA-damage responses as well as in signaling processes leading to activation of the transcription factor NF-kappa-B. Linear polymer chains formed via attachment by the initiator Met lead to cell signaling. Ubiquitin is usually conjugated to Lys residues of target proteins, however, in rare cases, conjugation to Cys or Ser residues has been observed. When polyubiquitin is free (unanchored-polyubiquitin), it also has distinct roles, such as in activation of protein kinases, and in signaling. Similarity: Belongs to the ubiquitin family. Contains 3 ubiquitin-like domains.

Cellular localization Cell Membrane, Cytoplasmic and Nuclear

Images



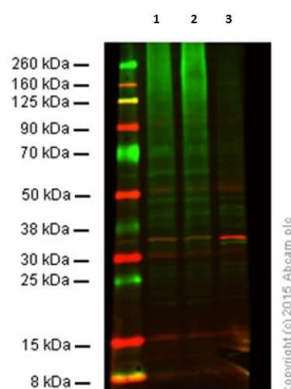
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [Ubi-1] (ab7254)

Image from Ohmi K et al., Plos One. 2011;6(11):e27461. Fig 7.; doi: 10.1371/journal.pone.0027461 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

Immunohistochemical staining of ubiquitin in a 7 months-old MPS IIIA mouse brain (PFA fixed) using ab7254.

Sections, 40 μ m thick, were cut sagittally on a vibratome. Slices were permeabilized in 1% triton X-100/PBS for 1 hour at room temperature. The samples were placed on vectabond-coated glass slides and dried overnight. They were then rehydrated in PBS and washed in ice-cold methanol for 10 min.

The slices were washed in 0.3% H_2O_2 in methanol at room temperature and incubated sequentially with 0.5% normal donkey serum, ab7254 and biotin-labeled F(ab')₂ donkey secondary antibody in Tris-buffered saline (pH 7.5) containing 2% BSA and 0.02% Tween 20. The signal was detected with an ABC kit, visualized with diaminobenzidine and counterstained with hematoxylin.



Western blot - Anti-Ubiquitin antibody [Ubi-1] (ab7254)

All lanes : Anti-Ubiquitin antibody [Ubi-1] (ab7254) at 1/100 dilution

Lane 1 : HeLa cell lysate

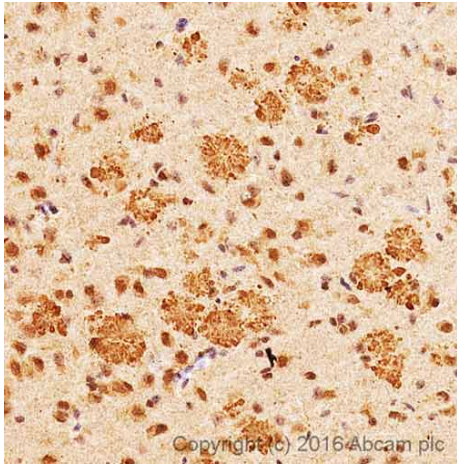
Lane 2 : HeLa cell lysate with MG132

Lane 3 : Mouse brain tissue lysate

Lysates/proteins at 20 μ g per lane.

Secondary

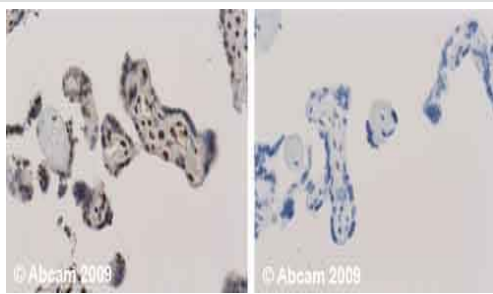
All lanes : Anti mouse IgG at 1/10000 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [Ubi-1] (ab7254)

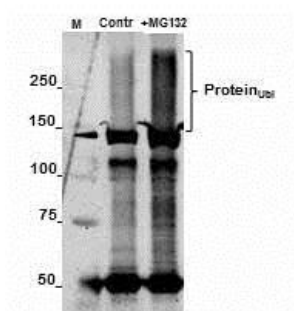
IHC image of ab7254 staining in mouse alzheimer brain formalin fixed paraffin embedded tissue section, using MOM detection kit, **ab127055**. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab7254, 5µg/ml, for 15 mins at room temperature. DAB was used as the chromogen (**ab103723**). The section was 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [Ubi-1] (ab7254)

Human normal placenta (**ab29745**). Staining is localised in the cytoplasm and in the nuclei. Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control. Sections were stained using an automated system DAKO Autostainer Plus, at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers citrate pH6.1 in a DAKO PT Link. Slides were peroxidase blocked in 3% H₂O₂ in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for mouse for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Western blot - Anti-Ubiquitin antibody [Ubi-1]
(ab7254)

Image courtesy of an anonymous Abreview.

HeLa cells were co-transfected with a plasmid expressing a target protein together with Ubi expressing vector for 24 hours and either left untreated (Contr) or were treated with 10 μ M MG-132 for 6 hours (+MG132). Then the protein of interest was pulled down using Flag agarose beads and and probed with ab7254 at a 1/2000 dilution. The secondary used was an Alexa-Fluor 680 conjugated goat anti-mouse polyclonal used at a 1/10000 dilution. The protein is known to be degraded through proteasome.

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors