abcam

Product datasheet

Anti-Ubiquitin antibody [EP296Y] ab33893

Recombinant RabMAb

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Overview

Product name	Anti-Ubiquitin antibody [EP296Y]
Description	Rabbit monoclonal [EP296Y] to Ubiquitin
Host species	Rabbit
Specificity	The antibody recognizes only mono-ubiquitinated molecules.
Tested applications	**Suitable for:** WB, IHC-P **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide within Human Ubiquitin aa 50-150 (C terminal). The exact sequence is proprietary.
Positive control	Hela cell lysate and human prostate adenocarcinoma tissue.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP296Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab33893 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
WB		1/1000 - 1/10000. Detects a band of approximately 8.5 kDa (predicted molecular weight: 8 kDa).
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

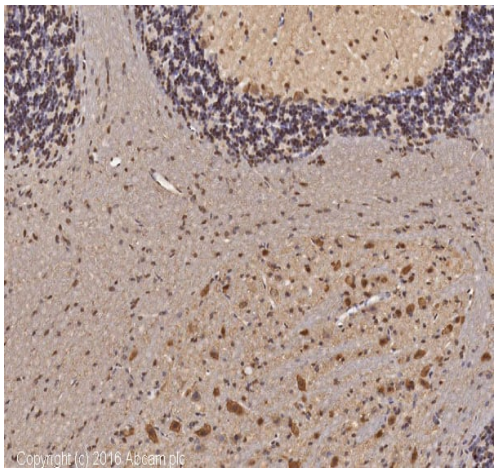
Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

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

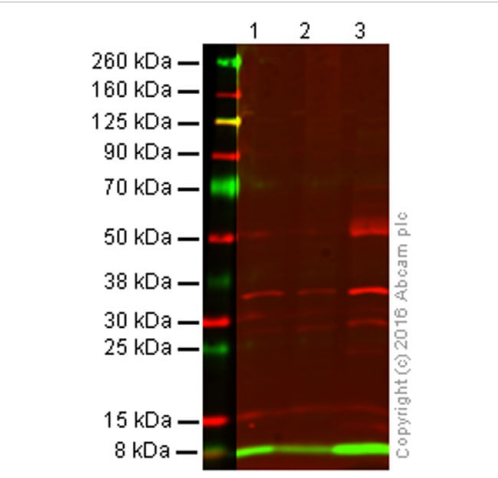
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EP296Y] (ab33893)

IHC image of Ubiquitin staining in mouse Alzheimer brain 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 ab33893, 1in500, 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.



Western blot - Anti-Ubiquitin antibody [EP296Y] (ab33893)

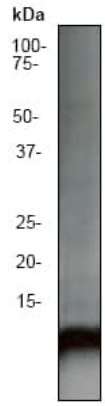
All lanes : Anti-Ubiquitin antibody [EP296Y] (ab33893) at 1/1000 dilution

Lane 1 : MCF-7 Whole cell lysate
Lane 2 : MCF-7 Whole cell lysate + (50 uM 90 min)
Lane 3 : Mouse brain tissue lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 8 kDa

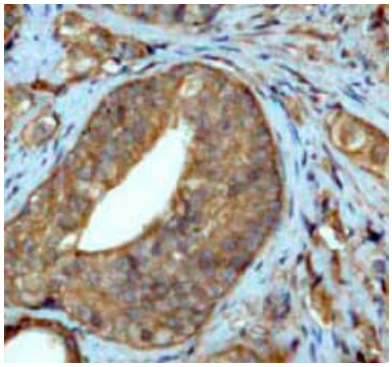
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. ab33893 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging using the Licor Odyssey CLx.



Western blot - Anti-Ubiquitin antibody [EP296Y] (ab33893)

Anti-Ubiquitin antibody [EP296Y] (ab33893) at 1/2000 dilution + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Predicted band size: 8 kDa
Observed band size: 8.5 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EP296Y] (ab33893)

ab33893 at a 1:250 dilution staining Ubiquitin in human prostate adenocarcinoma using Immunohistochemistry, Paraffin Embedded Tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-Ubiquitin antibody [EP296Y] (ab33893)

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