

## Product datasheet

# Anti-Daxx antibody [E94] ab32140

**KO VALIDATED** Recombinant RabMAB

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### Overview

<b>Product name</b>	Anti-Daxx antibody [E94]
<b>Description</b>	Rabbit monoclonal [E94] to Daxx
<b>Host species</b>	Rabbit
<b>Specificity</b>	Not widely detected in Mouse and Rat
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, Flow Cyt, Flow Cyt (Intra), ICC/IF <b>Unsuitable for:</b> IP
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide within Human Daxx aa 250-350. The exact sequence is proprietary.
<b>Positive control</b>	WB: HeLa and HAP1 cell lysates. IHC-P: Human stomach adenocarcinoma. IF/ICC: HeLa cell line.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	E94

Isotype

IgG

## Applications

### The Abpromise guarantee

Our [Abpromise guarantee](#) covers the use of ab32140 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		1/5000. Detects a band of approximately 100 kDa (predicted molecular weight: 81 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt		1/100. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
Flow Cyt (Intra)		1/100. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50.

### Application notes

Is unsuitable for IP.

## Target

### Function

Transcription corepressor known to repress transcriptional potential of several sumoylated transcription factors. Down-regulates basal and activated transcription. Its transcription repressor activity is modulated by recruiting it to subnuclear compartments like the nucleolus or PML/POD/ND10 nuclear bodies through interactions with MCSR1 and PML, respectively. Seems to regulate transcription in PML/POD/ND10 nuclear bodies together with PML and may influence TNFRSF6-dependent apoptosis thereby. Inhibits transcriptional activation of PAX3 and ETS1 through direct protein-protein interactions. Modulates PAX5 activity; the function seems to involve CREBBP. Acts as an adapter protein in a MDM2-DAXX-USP7 complex by regulating the RING-finger E3 ligase MDM2 ubiquitination activity. Under non-stress condition, in association with the deubiquitinating USP7, prevents MDM2 self-ubiquitination and enhances the intrinsic E3 ligase activity of MDM2 towards TP53, thereby promoting TP53 ubiquitination and subsequent proteasomal degradation. Upon DNA damage, its association with MDM2 and USP7 is disrupted, resulting in increased MDM2 autoubiquitination and consequently, MDM2 degradation, which leads to TP53 stabilization. Acts as histone chaperone that facilitates deposition of histone H3.3. Acts as targeting component of the chromatin remodeling complex ATRX:DAXX which has ATP-dependent DNA translocase activity and catalyzes the replication-independent deposition of histone H3.3 in pericentric DNA repeats outside S-phase and telomeres, and the in vitro remodeling of H3.3-containing nucleosomes. Does not affect the ATPase activity of ATRX but alleviates its transcription repression activity. Upon neuronal activation associates with regulatory elements of selected immediate early genes where it promotes deposition of histone H3.3 which may be linked to transcriptional induction of these genes. Required for the recruitment of histone

H3.3:H4 dimers to PML-nuclear bodies (PML-NBs); the process is independent of ATRX and facilitated by ASF1A; PML-NBs are suggested to function as regulatory sites for the incorporation of newly synthesized histone H3.3 into chromatin. In case of overexpression of centromeric histone variant CENPA (as found in various tumors) is involved in its mislocalization to chromosomes; the ectopic localization involves a heterotypic tetramer containing CENPA, and histones H3.3 and H4 and decreases binding of CTCF to chromatin. Proposed to mediate activation of the JNK pathway and apoptosis via MAP3K5 in response to signaling from TNFRSF6 and TGFBR2. Interaction with HSPB1/HSP27 may prevent interaction with TNFRSF6 and MAP3K5 and block DAXX-mediated apoptosis. In contrast, in lymphoid cells JNC activation and TNFRSF6-mediated apoptosis may not involve DAXX. Shows restriction activity towards human cytomegalovirus (HCMV).

**Tissue specificity**

Ubiquitous.

**Sequence similarities**

Belongs to the DAXX family.

**Domain**

The Sumo interaction motif mediates Sumo binding, and is required both for sumoylation and binding to sumoylated targets.

**Post-translational modifications**

Sumoylated with SUMO1 on multiple lysine residues.

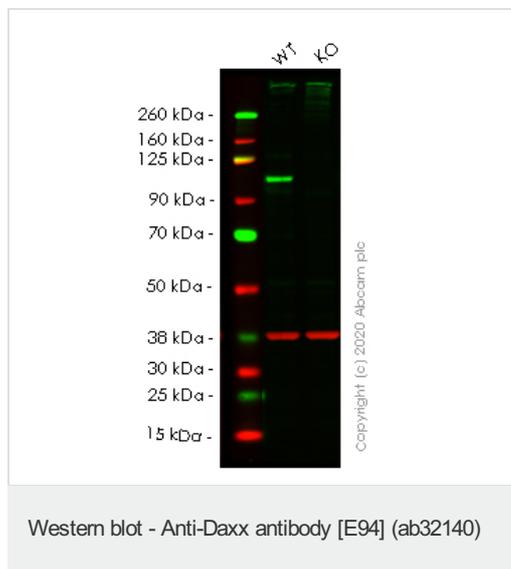
Phosphorylated by HIPK1 upon glucose deprivation.

Polyubiquitinated; which is promoted by CUL3 and SPOP and results in proteasomal degradation. Ubiquitinated by MDM2; inducing its degradation. Deubiquitinated by USP7; leading to stabilize it.

**Cellular localization**

Nucleus. Diffuse nuclear distribution pattern and no comparable dot-like accumulation of isoform 1 and Cytoplasm. Nucleus, nucleoplasm. Nucleus, PML body. Nucleus, nucleolus. Chromosome, centromere. Dispersed throughout the nucleoplasm, in PML/POD/ND10 nuclear bodies, and in nucleoli (Probable). Colocalizes with histone H3.3, ATRX, HIRA and ASF1A at PML-nuclear bodies (PubMed:12953102, PubMed:14990586, PubMed:23222847, PubMed:24200965). Colocalizes with a subset of interphase centromeres, but is absent from mitotic centromeres (PubMed:9645950). Detected in cytoplasmic punctate structures (PubMed:11842083). Translocates from the nucleus to the cytoplasm upon glucose deprivation or oxidative stress (PubMed:12968034). Colocalizes with RASSF1 in the nucleus (PubMed:18566590). Colocalizes with USP7 in nucleoplasm with accumulation in speckled structures (PubMed:16845383).

**Images**



**All lanes :** Anti-Daxx antibody [E94] (ab32140) at 1/5000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** DAXX knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

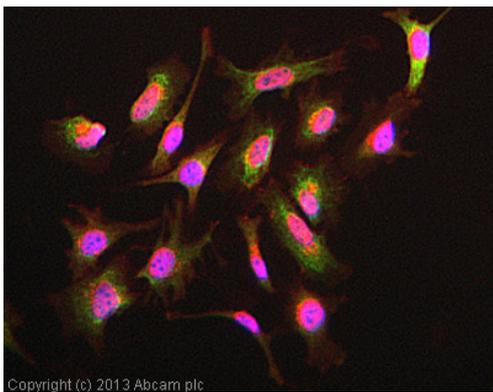
Performed under reducing conditions.

**Predicted band size:** 81 kDa

**Observed band size:** 100 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab32140 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

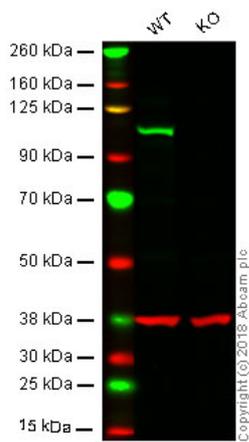
ab32140 was shown to react with Daxx in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265233 (knockout cell lysate ab257408) was used. Wild-type HeLa and DAXX knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32140 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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Immunocytochemistry/ Immunofluorescence - Anti-Daxx antibody [E94] (ab32140)

ab32140 stained HeLa cells. The cells were 4% formaldehyde fixed for 10 minutes at room temperature and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32140 at 10µg/ml) overnight at +4°C. The secondary antibody (pseudo-colored green) was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) used at a 1/1000 dilution for 1 hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1 hour at room temperature.



Western blot - Anti-Daxx antibody [E94] (ab32140)

**All lanes :** Anti-Daxx antibody [E94] (ab32140)

**Lane 1 :** Wild-type HAP1 whole cell lysate

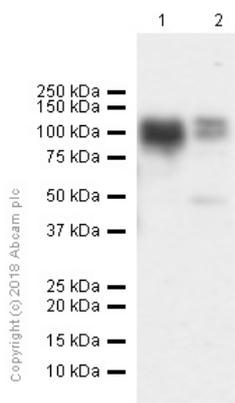
**Lane 2 :** DAXX knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size:** 81 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab32140 observed at 100 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab32140 was shown to specifically react with Daxx in wild-type HAP1 cells as signal was lost in DAXX knockout cells. Wild-type and DAXX knockout samples were subjected to SDS-PAGE. ab32140 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Daxx antibody [E94] (ab32140)

**All lanes :** Anti-Daxx antibody [E94] (ab32140) at 0.271 µg/ml

**Lane 1 :** PC-12(Rat adrenal gland pheochromocytoma) whole cell lysate

**Lane 2 :** NIH/3T3(Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

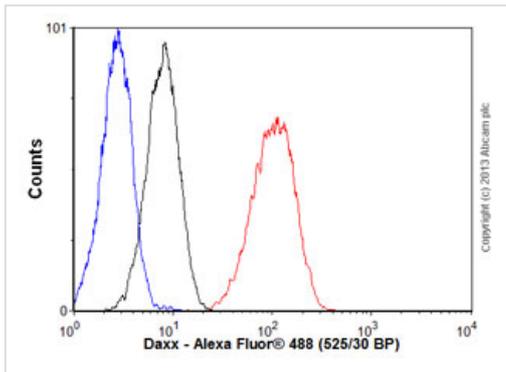
**Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 81 kDa

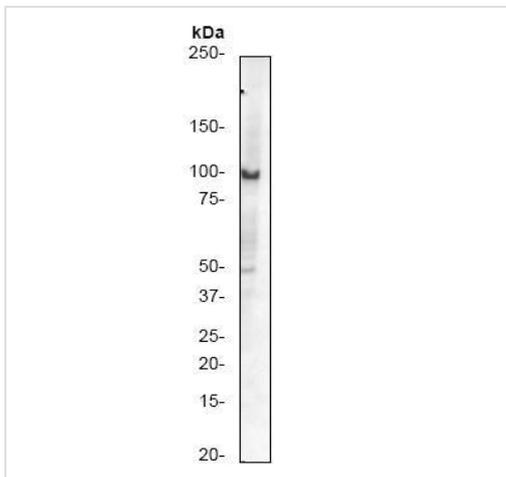
**Exposure time:** 29 seconds

Blocking and diluting buffer: 5% NFDm/TBST.



Flow Cytometry (Intracellular) - Anti-Daxx antibody [E94] (ab32140)

Overlay histogram showing HeLa cells stained with ab32140 (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 (ab32140, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



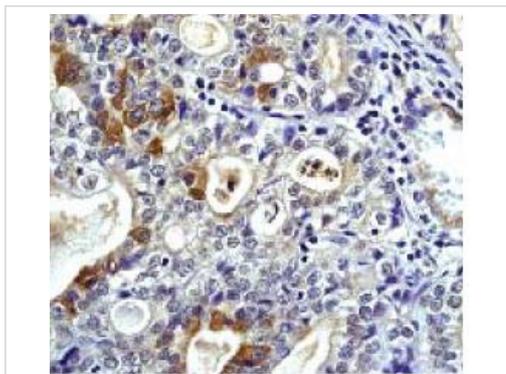
Western blot - Anti-Daxx antibody [E94] (ab32140)

Anti-Daxx antibody [E94] (ab32140) at 1/5000 dilution + HeLa cell lysate

**Predicted band size:** 81 kDa

**Observed band size:** 100 kDa

**Additional bands at:** 48 kDa. We are unsure as to the identity of these extra bands.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Daxx antibody [E94] (ab32140)

Ab32140, at a dilution of 1/50, staining Daxx in paraffin embedded human stomach adenocarcinoma tissue by Immunohistochemistry.

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

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Daxx antibody [E94] (ab32140)

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

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