abcam

Product datasheet

Anti-Daxx antibody [E94] ab32140

KO VALIDATED Recombinant RabMAb

1 Abreviews 16 References 11 Images

Overview

Product name	Anti-Daxx antibody [E94]
Description	Rabbit monoclonal [E94] to Daxx
Host species	Rabbit
Specificity	Not widely detected in Mouse and Rat
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt (Intra), ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Daxx aa 250-350. The exact sequence is proprietary.
Positive control	WB: HeLa, HAP1, wild type HTC116, THP-1, K562 and Wild-type MCF7 cell lysates. IHC-P: Human stomach adenocarcinoma. IF/ICC: HeLa cell line.
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: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	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 (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50.

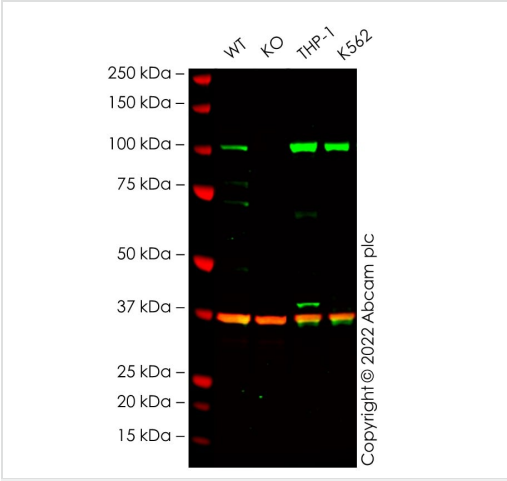
Application notes Is unsuitable for IP.

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 nucleoplasma with accumulation in speckled structures (PubMed:16845383).

Images



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

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

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : DAXX knockout MCF7 cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : K562 cell lysate

Lysates/proteins at 20 µg per lane.

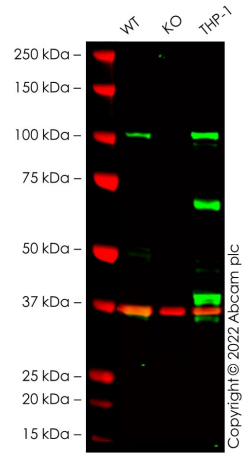
Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 81 kDa

Observed band size: 105 kDa

False colour image of Western blot: Anti-Daxx antibody [E94] staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32140 was shown to bind specifically to Daxx. A band was observed at 105 kDa in wild-type MCF7 cell lysates with no signal observed at this size in DAXX knockout cell line. To generate this image, wild-type and DAXX knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



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

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

Lane 1 : Wild-type A549 cell lysate
Lane 2 : DAXX knockout A549 cell lysate
Lane 3 : THP-1 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

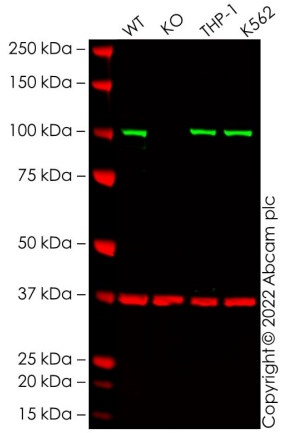
Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 105 kDa

False colour image of Western blot: Anti-Daxx antibody [E94] staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32140 was shown to bind

specifically to Daxx. A band was observed at 105 kDa in wild-type A549 cell lysates with no signal observed at this size in DAXX knockout cell line.

To generate this image, wild-type and DAXX knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



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

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

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : DAXX knockout HCT 116 cell lysate
Lane 3 : THP-1 cell lysate
Lane 4 : K562 cell lysate

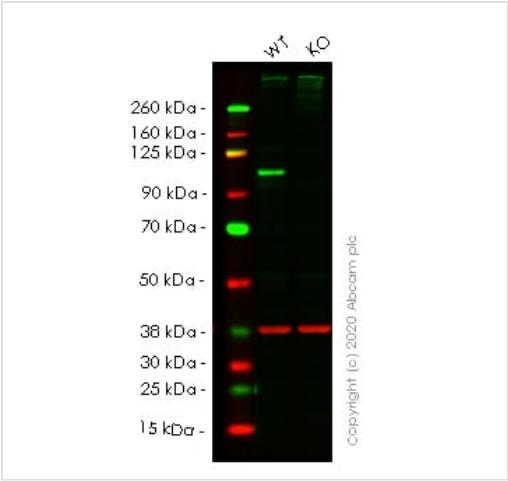
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 100 kDa

False colour image of Western blot: Anti-Daxx antibody [E94] staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32140 was shown to bind specifically to Daxx. A band was observed at 100 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in DAXX knockout cell line. To generate this image, wild-type and DAXX knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG

H&L 680RD at 1/20000 dilution.



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

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

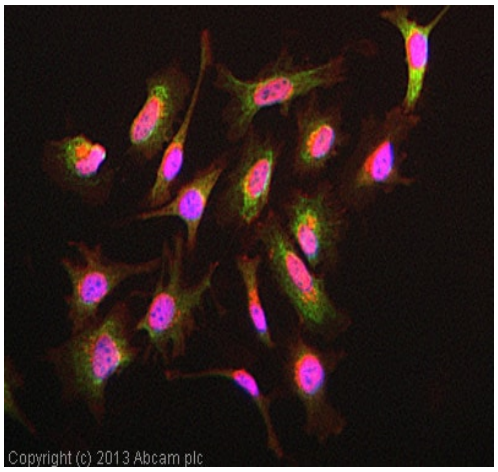
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa
Observed band size: 100 kDa

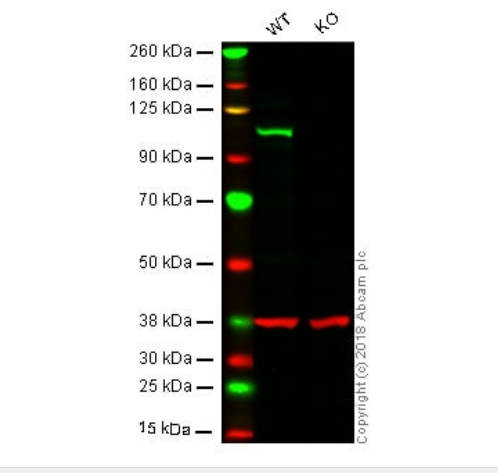
Lanes 1- 2: Merged signal (red and green). Green - ab32140 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

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.



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 1hour 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 1hour at room temperature. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.



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

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

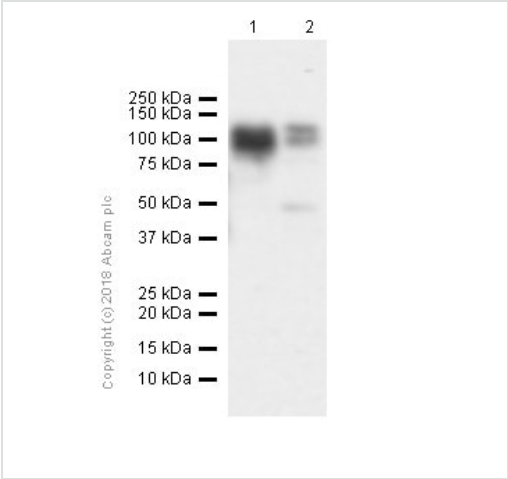
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : DAXX knockout HAP1 whole cell lysate

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

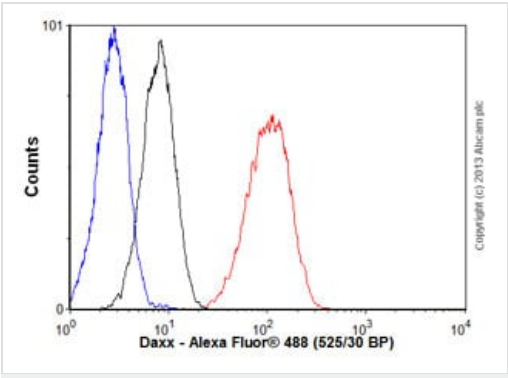
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 81 kDa

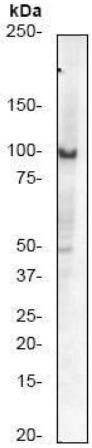
Exposure time: 29 seconds

Blocking and diluting buffer: 5% NFDM/TBST.



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

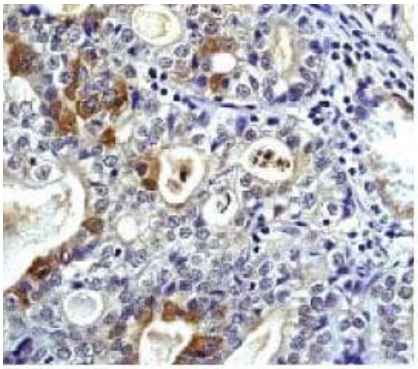
Overlay histogram showing HeLa cells stained with ab32140 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions 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/1x10^6 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

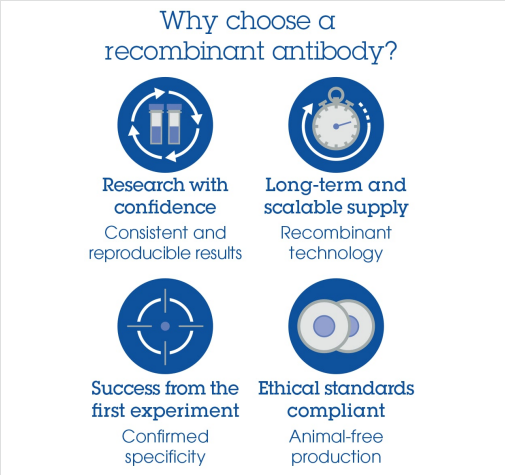
Predicted band size: 81 kDa
Observed band size: 100 kDa
Additional bands at: 48 kDa. We are unsure as to the identity of these extra bands.



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.



Anti-Daxx antibody [E94] (ab32140)

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