abcam

Product datasheet

Anti-DDX6 antibody ab40684

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Overview

Product name	Anti-DDX6 antibody
Description	Rabbit polyclonal to DDX6
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Chicken, Guinea pig
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 450 to the C-terminus of Human DDX6.Read Abcam's proprietary immunogen policy(Peptide available as ab41593.)
Positive control	This antibody gave a positive signal in the following whole cell lysates: HEK 293, A431, Jurkat. This antibody gave a positive result in IHC in the following FFPE tissue: Human skeletal muscle.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab40684** 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		1/250. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
IHC-P		Use a concentration of 5 µg/ml.
ICC/IF		Use a concentration of 1 µg/ml.

Target

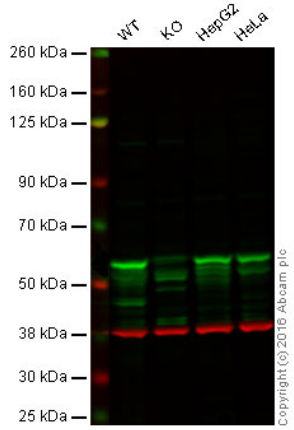
Relevance

DEAD box proteins, characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD), are putative RNA helicases. They are implicated in a number of cellular processes involving alteration of RNA secondary structure such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. Based on their distribution patterns, some members of this family are believed to be involved in embryogenesis, spermatogenesis and cellular growth and division. In the process of mRNA degradation, DDX6 may play a role in mRNA decapping. It forms a complex with DCP1A, DCP2, EDC3 and EDC4/HEDLS.

Cellular localization

Cytoplasm; P-body. Note: Processing bodies (PB).

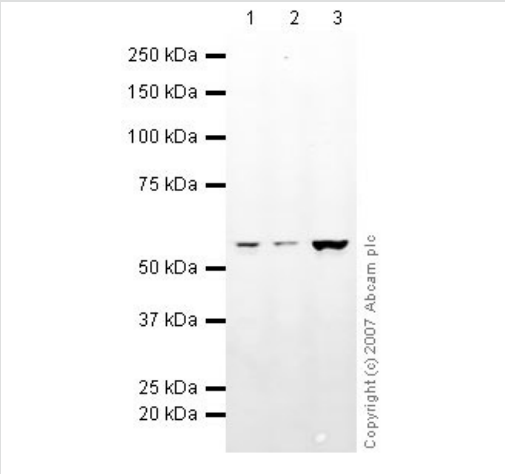
Images



Western blot - Anti-DDX6 antibody (ab40684)

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: DDX6 knockout HAP1 cell lysate (40 µg)
Lane 3: HepG2 cell lysate (20 µg)
Lane 4: HeLa cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab40684 observed at 55 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab40684 was shown not to react with DDX6 when DDX6 knockout samples were used. Wild-type and DDX6 knockout samples were subjected to SDS-PAGE. Ab40684 and ab8245 (loading control to GAPDH) were diluted at 1/100 and 1:10,000 dilution respectively and incubated overnight at 4C. Blots were developed with IRDye® 800CW Goat anti-Rabbit IgG (H + L) ab216773 and IRDye® 680 Goat anti-Mouse IgG (H + L) ab216776 secondary antibodies at 1:10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-DDX6 antibody (ab40684)

All lanes : Anti-DDX6 antibody (ab40684) at 1 µg/ml

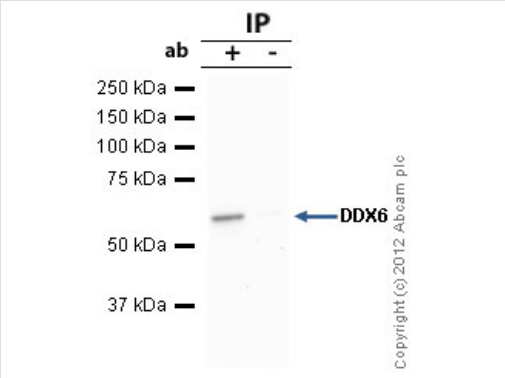
Lane 1 : HEK293 Human embryonic kidney cell line Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

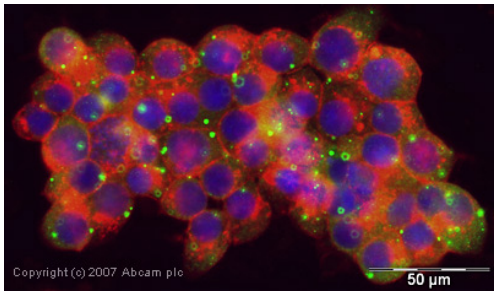
Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 54 kDa



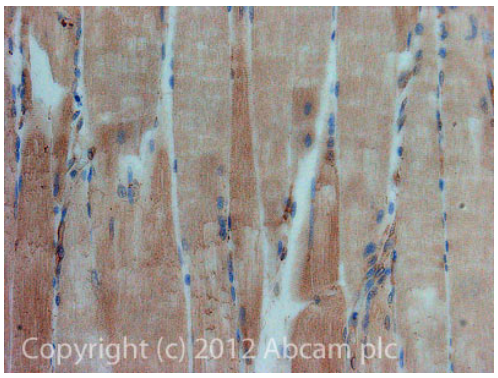
Immunoprecipitation - Anti-DDX6 antibody (ab40684)

DDX6 was immunoprecipitated using 0.5mg Jurkat whole cell extract, 5µg of Rabbit polyclonal to DDX6 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Jurkat whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab40684.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).
Band: 54kDa: DDX6



Immunocytochemistry/ Immunofluorescence - Anti-DDX6 antibody (ab40684)

ICC/IF image of ab40684 stained human HEK 293 cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab40684, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DDX6 antibody (ab40684)

IHC image of DDX6 staining in Human skeletal muscle formalin fixed paraffin embedded tissue section, performed on a Leica BondTM 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 ab40684, 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.

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