abcam

Product datasheet

Anti-DDB2 antibody [EPR9811] ab181136





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Overview

Product name Anti-DDB2 antibody [EPR9811]

Description Rabbit monoclonal [EPR9811] to DDB2

Host species Rabbit

Tested applications Suitable for: WB, IHC-P

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Human

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen**

Positive control WB: HeLa, SH-SY5Y and Raji cell lysates; IHC-P: Human stomach carcinoma 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 $\mathsf{RabMAb}^{\texttt{®}}$ 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number **EPR9811**

Isotype ΙgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab181136 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 45 kDa (predicted molecular weight: 48 kDa).
IHC-P		1/2500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/50 - 1/100.

Application notes

Is unsuitable for Flow Cyt or ICC/IF.

Target

Function

Required for DNA repair. Binds to DDB1 to form the UV-damaged DNA-binding protein complex (the UV-DDB complex). The UV-DDB complex may recognize UV-induced DNA damage and recruit proteins of the nucleotide excision repair pathway (the NER pathway) to initiate DNA repair. The UV-DDB complex preferentially binds to cyclobutane pyrimidine dimers (CPD), 6-4 photoproducts (6-4 PP), apurinic sites and short mismatches. Also appears to function as the substrate recognition module for the DCX (DDB1-CUL4-X-box) E3 ubiquitin-protein ligase complex DDB1-CUL4-ROC1 (also known as CUL4-DDB-ROC1 and CUL4-DDB-RBX1). The DDB1-CUL4-ROC1 complex may ubiquitinate histone H2A, histone H3 and histone H4 at sites of UV-induced DNA damage. The ubiquitination of histones may facilitate their removal from the nucleosome and promote subsequent DNA repair. The DDB1-CUL4-ROC1 complex also ubiquitinates XPC, which may enhance DNA-binding by XPC and promote NER. Isoform D1 and isoform D2 inhibit UV-damaged DNA repair.

Tissue specificity

Ubiquitously expressed; with highest levels in corneal endothelium and lowest levels in brain. Isoform D1 is highly expressed in brain and heart. Isoform D2, isoform D3 and isoform D4 are weakly expressed.

Pathway

Protein modification; protein ubiquitination.

Involvement in disease

Defects in DDB2 are a cause of xeroderma pigmentosum complementation group E (XP-E) [MIM:278740]; also known as xeroderma pigmentosum V (XP5). XP-E is a rare human autosomal recessive disease characterized by solar sensitivity, high predisposition for developing cancers on areas exposed to sunlight and, in some cases, neurological abnormalities.

Sequence similarities

Belongs to the WD repeat DDB2/WDR76 family.

Contains 5 WD repeats.

Domain

The DWD box is required for interaction with DDB1.

Post-translational

Phosphorylation by ABL1 negatively regulate UV-DDB activity.

modifications

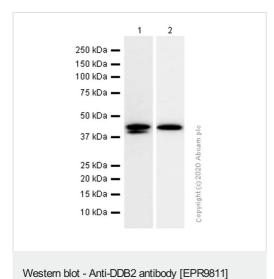
Ubiquitinated by CUL4A in response to UV irradiation. Ubiquitination appears to both impair DNA-binding and promotes ubiquitin-dependent proteolysis. Degradation of DDB2 at sites of DNA damage may be a prerequisite for their recognition by XPC and subsequent repair. CUL4A-

mediated degradation appears to be promoted by ABL1.

Images

(ab181136)

(ab181136)



Lane 1: Anti-DDB2 antibody [EPR9811] (ab181136) at 1/2000 dilution

Lane 2: Anti-DDB2 antibody [EPR9811] (ab181136) at 1/2000 dilution (Purified)

Lane 1 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysate

Lane 2 : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate

Lysates/proteins at 15 µg per lane.

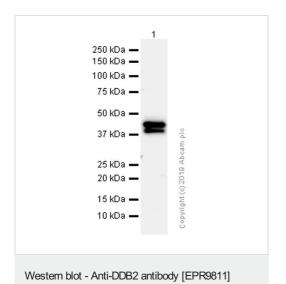
Secondary

Lane 1 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Lane 2 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 48 kDa

40kDa band might be an isomer of DNA damage-binding protein 2 (Isomer D3).



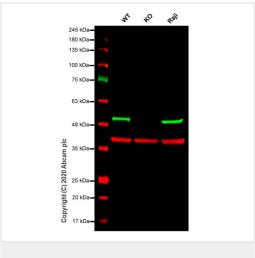
Anti-DDB2 antibody [EPR9811] (ab181136) at 1/1000 dilution + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 μg

Secondary

Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 48 kDa

40kDa band might be an isomer of DNA damage-binding protein 2 (Isomer D3).



Western blot - Anti-DDB2 antibody [EPR9811] (ab181136)

All lanes : Anti-DDB2 antibody [EPR9811] (ab181136) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: DDB2 knockout HeLa cell lysate

Lane 3: Raji cell lysate

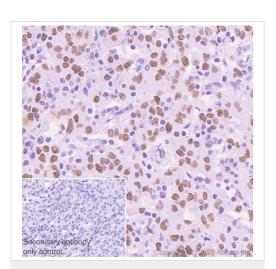
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 48 kDa **Observed band size:** 48 kDa

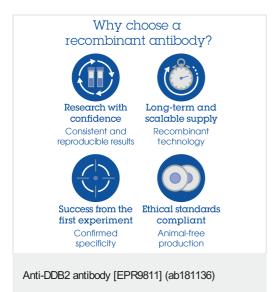
Lanes 1-3: Merged signal (red and green). Green - ab181136 observed at 48 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab181136 Anti-DDB2 antibody [EPR9811] was shown to specifically react with DDB2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265115 (knockout cell lysate ab257177) was used. Wild-type and DDB2 knockout samples were subjected to SDS-PAGE. ab181136 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DDB2 antibody
[EPR9811] (ab181136)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human stomach carcinoma tissue sections labeling DDB2 with Purified ab181136 at 1/2500 dilution (0.25 µg/mL). Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



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