abcam

Product datasheet

Anti-hHR23b antibody [2857D7a] ab70602

KO VALIDATED

1 References 4 Images

Overview

Product name	Anti-hHR23b antibody [2857D7a]
Description	Mouse monoclonal [2857D7a] to hHR23b
Host species	Mouse
Tested applications	**Suitable for:** IHC-P, WB, Dot blot
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment derived from internal sequence of human hHR23b.
Positive control	WB: HeLa, MCF7 and SW620 cell lysates; Recombinant human hHR23b. IHC-P: Human cervival carcinoma tissue.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.05% Sodium azide Constituents: 1% BSA, PBS
Purity	Protein G purified
Purification notes	ab70602 was purified using protein G column chromatography from culture supernatant of hybridoma cultured in a medium containing bovine IgG-depleted (approximately 95%) fetal bovine serum and filtered through a 0.22µm membrane.
Clonality	Monoclonal
Clone number	2857D7a
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab70602** 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
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use a concentration of 0.2 - 2 µg/ml. Detects a band of approximately 54 kDa (predicted molecular weight: 43 kDa).
Dot blot		Use at an assay dependent concentration.

Target

Function

Multiubiquitin chain receptor involved in modulation of proteasomal degradation. Binds to polyubiquitin chains. Proposed to be capable to bind simultaneously to the 26S proteasome and to polyubiquitinated substrates and to deliver ubiquitinated proteins to the proteasome. May play a role in endoplasmatic reticulum-associated degradation (ERAD) of misfolded glycoproteins by association with PNGase and delivering deglycosylated proteins to the proteasome.
Involved in global genome nucleotide excision repair (GG-NER) by acting as component of the XPC complex. Cooperatively with CETN2 appears to stabilize XPC. May protect XPC from proteasomal degradation.
The XPC complex is proposed to represent the first factor bound at the sites of DNA damage and together with other core recognition factors, XPA, RPA and the TFIIH complex, is part of the pre-incision (or initial recognition) complex. The XPC complex recognizes a wide spectrum of damaged DNA characterized by distortions of the DNA helix such as single-stranded loops, mismatched bubbles or single stranded overhangs. The orientation of XPC complex binding appears to be crucial for inducing a productive NER. XPC complex is proposed to recognize and to interact with unpaired bases on the undamaged DNA strand which is followed by recruitment of the TFIIH complex and subsequent scanning for lesions in the opposite strand in a 5'-to-3' direction by the NER machinery. Cyclobutane pyrimidine dimers (CPDs) which are formed upon UV-induced DNA damage esacpe detection by the XPC complex due to a low degree of structural perurbation. Instead they are detected by the UV-DDB complex which in turn recruits and cooperates with the XPC complex in the respective DNA repair. In vitro, the XPC:RAD23B dimer is sufficient to initiate NER; it preferentially binds to cisplatin and UV-damaged double-stranded DNA and also binds to a variety of chemically and structurally diverse DNA adducts. XPC:RAD23B contacts DNA both 5' and 3' of a cisplatin lesion with a preference for the 5' side. XPC:RAD23B induces a bend in DNA upon binding. XPC:RAD23B stimulates the activity of DNA glycosylases TDG and SMUG1.

Sequence similarities

Belongs to the RAD23 family.
Contains 1 STI1 domain.
Contains 2 UBA domains.
Contains 1 ubiquitin-like domain.

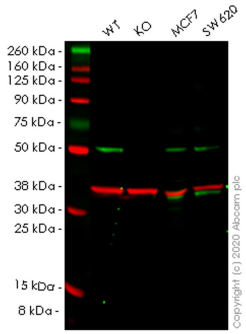
Domain

The ubiquitin-like domain mediates interaction with ATXN3.

Cellular localization

Nucleus. Cytoplasm. The intracellular distribution is cell cycle dependent. Localized to the nucleus and the cytoplasm during G1 phase. Nuclear levels decrease during S-phase; upon entering mitosis, relocalizes in the cytoplasm without association with chromatin.

Images



Western blot - Anti-hHR23b antibody [2857D7a] (ab70602)

All lanes : Anti-hHR23b antibody [2857D7a] (ab70602) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RAD23B knockout HeLa cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : SW620 cell lysate

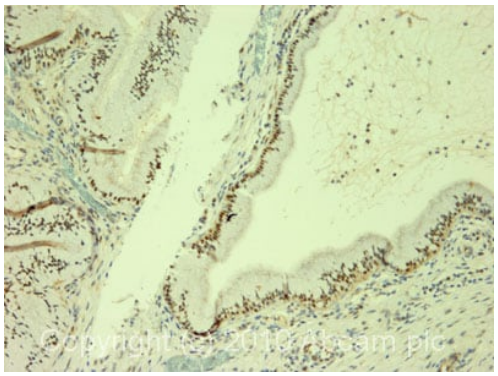
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Predicted band size: 43 kDa
Observed band size: 50 kDa
why is the actual band size different from the predicted?

Lanes 1-4: Merged signal (red and green). Green - ab70602 observed at 50 kDa. Red - loading control ab181602 observed at 36 kDa.

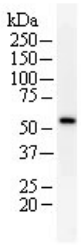
ab70602 Anti-hHR23b antibody [2857D7a] was shown to specifically react with hHR23b in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264961 (knockout cell lysate ab258623) was used. Wild-type and hHR23b knockout samples were subjected to SDS-PAGE. ab70602 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) were incubated at room temperature for 2.5 hours at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-hHR23b antibody [2857D7a] (ab70602)

IHC image of ab70602 staining in human normal cervical carcinoma 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 ab70602, 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.

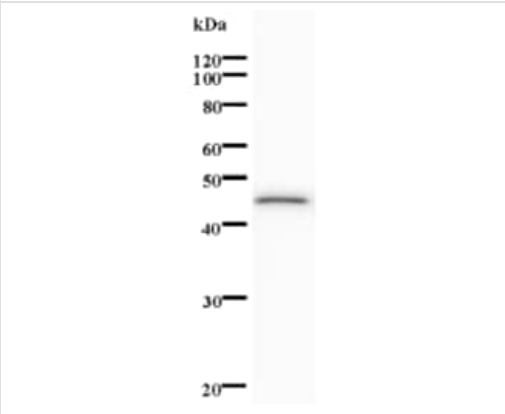
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-hHR23b antibody [2857D7a] (ab70602)

Anti-hHR23b antibody [2857D7a] (ab70602) at 0.2 µg/ml + Whole cell lysate from HeLa cells at 25 µg

Predicted band size: 43 kDa



Western blot - Anti-hHR23b antibody [2857D7a] (ab70602)

Anti-hHR23b antibody [2857D7a] (ab70602) at 0.2 µg/ml + immunising recombinant protein

Predicted band size: 43 kDa

Observed band size: 44 kDa why is the actual band size different from the predicted?

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