abcam

Product datasheet

Anti-HEXIM1 antibody ab25388

**** 2 Abreviews 28 References 4 Images

Overview

Product name Anti-HEXIM1 antibody

Description Rabbit polyclonal to HEXIM1

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, IP

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Dog

Immunogen Synthetic peptide conjugated to KLH derived from within residues 300 to the C-terminus of

Human HEXIM1. Read Abcam's proprietary immunogen policy (Peptide available as ab26978.)

Positive control ICC/IF: HeLa cells

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

1

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab25388 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)	Use a concentration of 1 µg/ml. Detects a band of approximately 54 kDa (predicted molecular weight: 41 kDa). Abcam recommends using milk (3%) as the blocking agent.
ICC/IF		Use a concentration of 1 µg/ml.
IP		Use at an assay dependent concentration.

Target

Tissue specificity

Function Transcriptional regulator which functions as a general RNA polymerase II transcription inhibitor. In

cooperation with 7SK snRNA sequesters P-TEFb in a large inactive 7SK snRNP complex preventing RNA polymerase II phosphorylation and subsequent transcriptional elongation. May

also regulate NF-kappa-B, ESR1, NR3C1 and CIITA-dependent transcriptional activity.

 $\label{thm:local_problem} \textbf{Ubiquitously expressed with higher expression in placenta. HEXIM1 and HEXIM2 are differentially}$

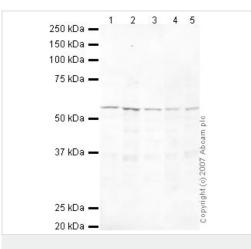
expressed. Expressed in endocrine tissues.

Sequence similarities Belongs to the HEXIM family.

Domain The coiled-coil domain mediates oligomerization.

Cellular localization Nucleus. Cytoplasm. Binds alpha-importin and is mostly nuclear.

Images



Western blot - Anti-HEXIM1 antibody (ab25388)

All lanes: Anti-HEXIM1 antibody (ab25388) at 1/250 dilution

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell

Lysate

Lane 2: Jurkat (Human T cell lymphoblast-like cell line) Whole Cell

Lysate

Lane 3: A431 (Human epithelial carcinoma cell line) Whole Cell

Lysate

Lane 4: MCF7 (Human breast adenocarcinoma cell line) Whole

Cell Lysate

Lane 5: SHSY-5Y (Human neuroblastoma cell line) Whole Cell

Lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 41 kDa Observed band size: 54 kDa

ab25388 recognizes a band at approximately 54 kDa that corresponds in size to that seen for HEXIM1. Although it has a predicted molecular weight of 41 kDa, it has been shown to migrate at a larger size of about 54-60 kDa (see Byers et al., J Biol Chem. 2005 Apr 22;280(16):16360-7 and Schulte et al., J. Biol. Chem., Vol. 280, (26): 24968-24977).

Immunoprecipitation - Anti-HEXIM1 antibody (ab25388)

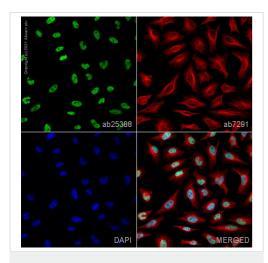
HEXIM1 - ChIP Grade was immunoprecipitated using 0.5mg Jurkat whole cell extract, 5µg of Rabbit polyclonal to HEXIM1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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 40μ I SDS loading buffer and incubated for 10min at 70^{o} C; 10μ I of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab25388.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

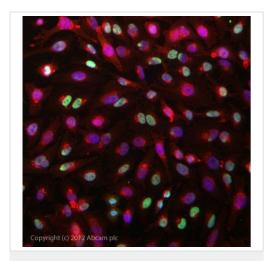
Band: 54kD: HEXIM1.



Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody (ab25388)

ab25388 staining HEXIM1 in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab25388 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor[®] 488), preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor[®] 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody (ab25388)

ICC/IF image of ab25388 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab25388 at 1 μ g/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) lgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 μ M.

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