abcam

Product datasheet

Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free ab272694



10 Images

Overview

Product name Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free

Description Rabbit monoclonal [EPR23430-12] to HEXIM1 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), IP, IHC-P, ChIP, WB

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: LNCaP, PC-12 whole cell lysates. IHC-P: Human cardiac muscle and testis tissue. ICC/IF:

HeLa and MEF cells. Flow Cyt (intra): HeLa cells. IP: HeLa (treated with 10 M MG-132 for 24

hours) and MEF whole cell lysates.

General notes ab272694 is the carrier-free version of ab240647.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR23430-12

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab272694 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
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 60 kDa (predicted molecular weight: 41 kDa).

Target

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.

Tissue specificity

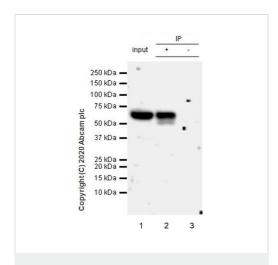
Ubiquitously expressed with higher expression in placenta. HEXIM1 and HEXIM2 are differentially

expressed. Expressed in endocrine tissues.

Sequence similarities Belongs to the HEXIM family.

Domain The coiled-coil domain mediates oligomerization.

Images



Immunoprecipitation - Anti-HEXIM1 antibody
[EPR23430-12] - BSA and Azide free (ab272694)

for 24 hours), whole cell lysate with <u>ab240647</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab240647</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) treated with 10 μM MG-132 for 24 hours, whole cell lysate 10 ug

HEXIM1 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) (treated with 10 µM MG-132

Lane 2: <u>ab240647</u> IP in HeLa treated with 10 µM MG-132 for 24

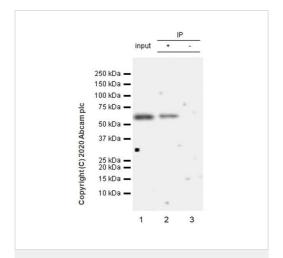
Lane 2: <u>ab240647</u> IP in HeLa treated with 10 μM MG-132 for 24 hours whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab240647</u> in HeLa treated with 10 μ M MG-132 for 24 hours whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



Immunoprecipitation - Anti-HEXIM1 antibody
[EPR23430-12] - BSA and Azide free (ab272694)

HEXIM1 was immunoprecipitated from 0.35 mg MEF (mouse embryonic fibroblast (immortalized)) whole cell lysate with ab240647 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab240647 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: MEF (mouse embryonic fibroblast (immortalized)) whole cell lysate 10 ug

Lane 2: ab240647 IP in MEF whole cell lysate

Lane 3: Rabbit monoclonal $\lg G$ (<u>ab172730</u>) instead of <u>ab240647</u> in MEF whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

Lysate were made freshly and used in IP test immediately to minimize protein degradation. Incubation for immunoprecipitation was carried out overnight at 4°C.

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).

ab240647 MERGED

DAPI Secondary antibody only control

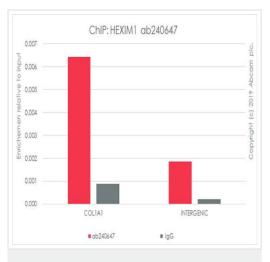
Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free (ab272694)

Immunofluorescent analysis of 100% methanol-fixed MEF cells labelling HEXIM1 with <u>ab240647</u> at 1/50 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 (Green). Confocal image showing nuclear staining in MEFs. <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u>

Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



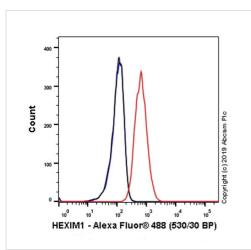
ChIP - Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free (ab272694)

Chromatin was prepared from MEF cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab 240647 (red), or 5 μ g of rabbit normal IgG <u>ab172730</u> (gray) and 20 μ I of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are from paper PMCID: PMC4103662

*https://www.abcam.com/resources? keywords=X%20ChIP%20protocol

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab240647</u>).



Flow Cytometry (Intracellular) - Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free (ab272694)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling HEXIM1 with ab240647 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

A Goat anti rabbit lgG (Alexa Fluor[®]488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HEXIM1 antibody

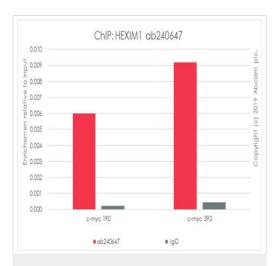
[EPR23430-12] - BSA and Azide free (ab272694)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling HEXIM1 with <u>ab240647</u> at 1/2000 dilution dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on human testis (PMID: 23300697). The section was incubated with <u>ab240647</u> for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

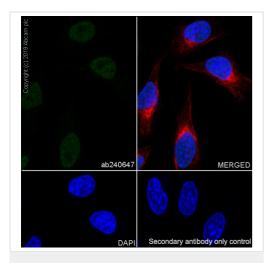
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



ChIP - Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free (ab272694)



Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody [EPR23430-12] - BSA and Azide free (ab272694)

Chromatin was prepared from LNCaP cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab240647 (red), or 5 μ g of rabbit normal lgG ab172730 (gray) and 20 μ l of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

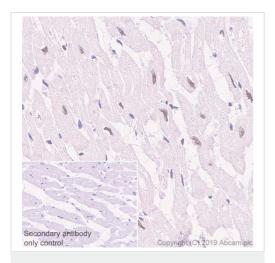
https://www.abcam.com/resources? keywords=X%20ChIP%20protocol

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).

Immunofluorescent analysis of 100% methanol-fixed HeLa cells labelling HEXIM1 with <u>ab240647</u> at 1/50 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) antibody at 1/1000 (Green). Confocal image showing nuclear staining in HeLa cells. <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HEXIM1 antibody

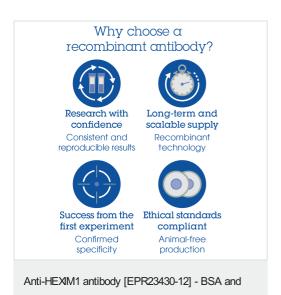
[EPR23430-12] - BSA and Azide free (ab272694)

Immunohistochemical analysis of paraffin-embedded Human cardiac muscle tissue labeling HEXIM1 with <u>ab240647</u> at 1/2000 dilution dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on human cardiac muscle (PMID: 23300697). The section was incubated with <u>ab240647</u> for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab240647).



Azide free (ab272694)

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