

## Product datasheet

# Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade ab240647

Recombinant RabMAb

11 Images

### Overview

<b>Product name</b>	Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade
<b>Description</b>	Rabbit monoclonal [EPR23430-12] to HEXIM1 - ChIP Grade
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P, ChIP
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	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.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal

Clone number                      EPR23430-12  
Isotype                                IgG

## Applications

**The Abpromise guarantee**            Our **Abpromise guarantee** covers the use of ab240647 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
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 60 kDa (predicted molecular weight: 41 kDa).
ICC/IF		1/50.
IP		1/30.
IHC-P		1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ChIP		Use 5 µg for 25 µg of chromatin.

## 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.

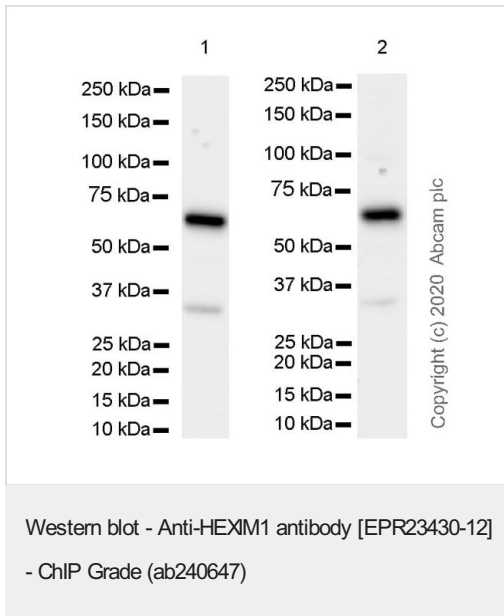
**Tissue specificity**                        Ubiquitously expressed with higher expression in placenta. HEXIM1 and HEXIM2 are differentially expressed. Expressed in endocrine tissues.

**Sequence similarities**                    Belongs to the HEXIM family.

**Domain**                                      The coiled-coil domain mediates oligomerization.

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

## Images



**All lanes :** Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647) at 1/1000 dilution

**Lane 1 :** LNCaP (human prostate carcinoma epithelial cell) whole cell lysate

**Lane 2 :** PC-12 (rat adrenal gland pheochromocytoma ) whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**Lane 1 :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated ([ab97051](#)))

**Lane 2 :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

**Predicted band size:** 41 kDa

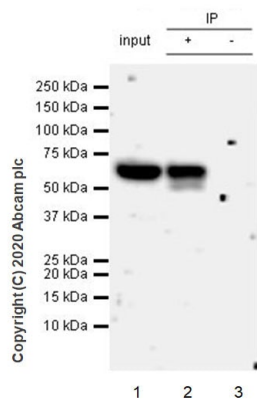
**Observed band size:** 60 kDa

Blocking and diluting buffer and concentration: 5% NFD/MTBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 28254838)

Lysates were made freshly and used in WB test immediately to minimize protein degradation.

Exposure time: 26 seconds.



Immunoprecipitation - Anti-HEXIM1 antibody  
[EPR23430-12] - ChIP Grade (ab240647)

HEXIM1 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) (treated with 10  $\mu$ M MG-132 for 24 hours), 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.

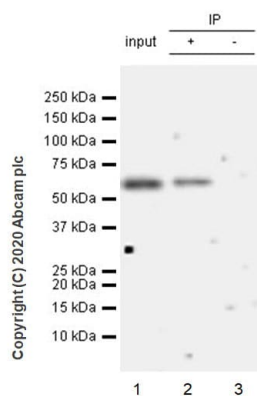
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) treated with 10  $\mu$ M MG-132 for 24 hours, whole cell lysate 10 ug

Lane 2: ab240647 IP in HeLa treated with 10  $\mu$ M MG-132 for 24 hours whole cell lysate

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab240647 in HeLa treated with 10  $\mu$ M MG-132 for 24 hours whole cell lysate

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

Exposure time: 3 minutes.



Immunoprecipitation - Anti-HEXIM1 antibody  
[EPR23430-12] - ChIP Grade (ab240647)

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

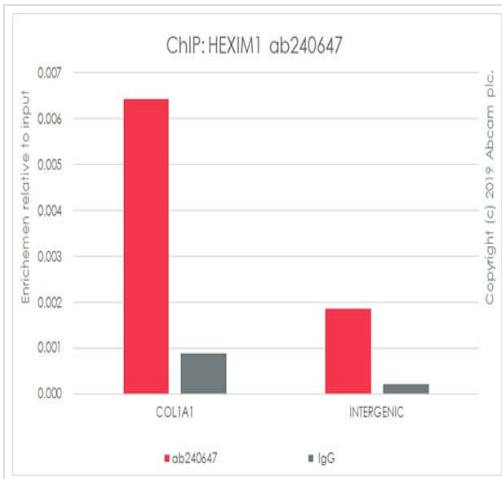
Lane 2: ab240647 IP in MEF whole cell lysate

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab240647 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.



ChIP - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

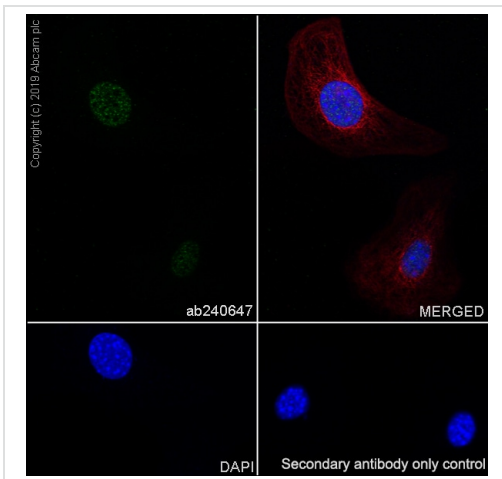
Chromatin was prepared from MEF cells according to the Abcam Dual-X-ChIP protocol\*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab 240647 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl 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 PMID: PMC4103662

\*[https://www.abcam.com/resources?](https://www.abcam.com/resources?keywords=X%20ChIP%20protocol)

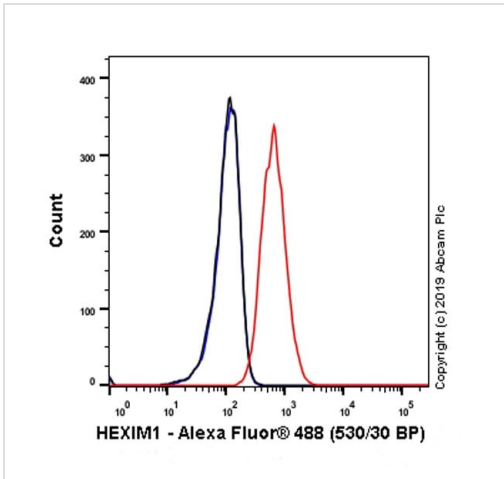
**keywords=X%20ChIP%20protocol**



Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

Immunofluorescent analysis of 100% methanol-fixed MEF cells labelling HEXIM1 with ab240647 at 1/50 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 (Green). Confocal image showing nuclear staining in MEFs. **ab195889** 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).

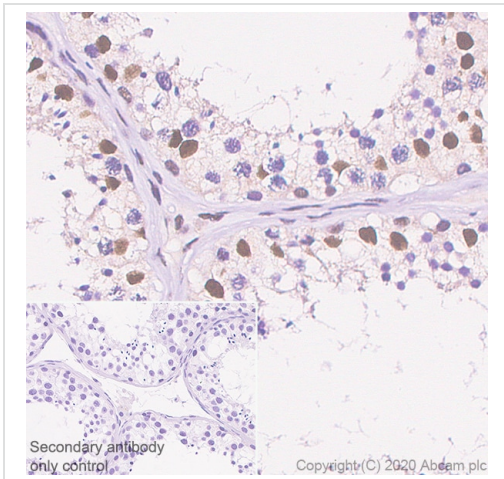
Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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 (0.1 ug) (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 IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

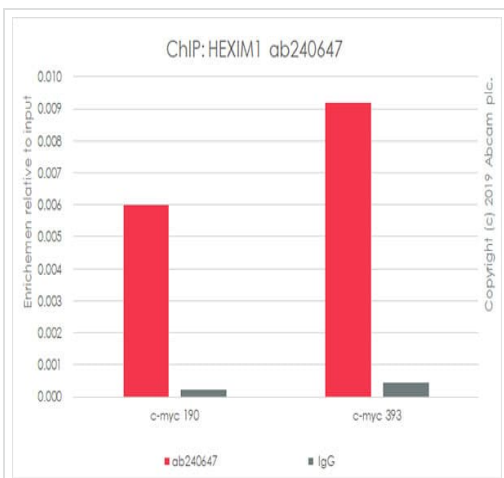


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling HEXIM1 with ab240647 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human testis (PMID: 23300697). The section was incubated with ab240647 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.



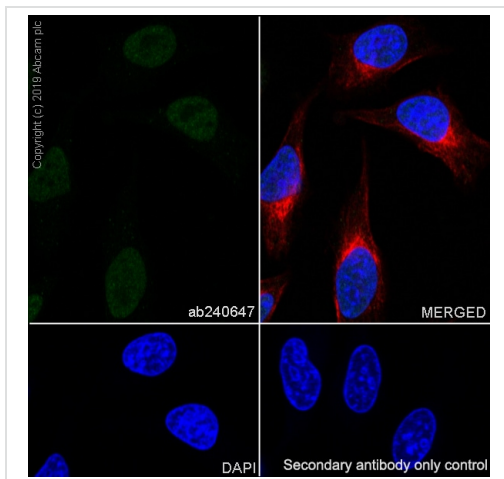
ChIP - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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 µg of chromatin, 5 µg of ab240647 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl 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?](https://www.abcam.com/resources?keywords=X%20ChIP%20protocol)

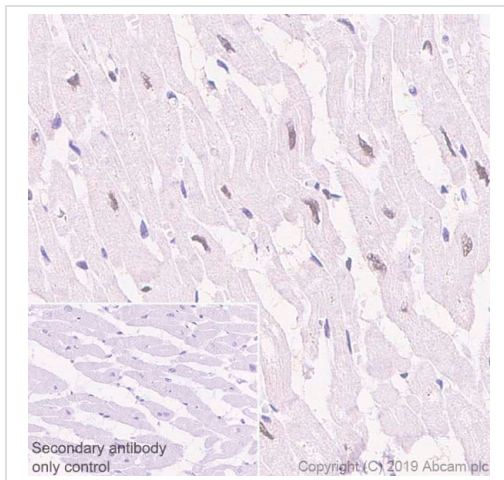
**keywords=X%20ChIP%20protocol**



Immunocytochemistry/ Immunofluorescence - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-HEXM1 antibody [EPR23430-12] - CHIP Grade  
(ab240647)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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