abcam

Product datasheet

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

ועלשעבע RabMAb

画像数 11

製品の概要

製品名 Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade

製品の詳細 Rabbit monoclonal [EPR23430-12] to HEXIM1 - ChIP Grade

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P, ChIP

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール 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.

特記事項 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**.

製品の特性

製品の状態

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR23430-12

アプリケーション

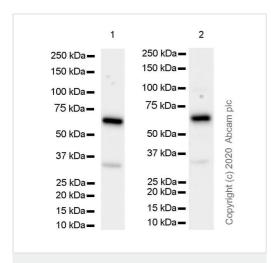
The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab240647の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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.

ターゲット情報

機能	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.	
組織特異性	Ubiquitously expressed with higher expression in placenta. HEXIM1 and HEXIM2 are differentially expressed. Expressed in endocrine tissues.	
配列類似性	Belongs to the HEXIM family.	
ドメイン	The coiled-coil domain mediates oligomerization.	
細胞内局在	Nucleus. Cytoplasm. Binds alpha-importin and is mostly nuclear.	

画像



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

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 lgG H&L (HRP) (ab97051) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051))

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

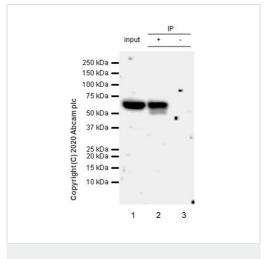
Predicted band size: 41 kDa Observed band size: 60 kDa

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

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.

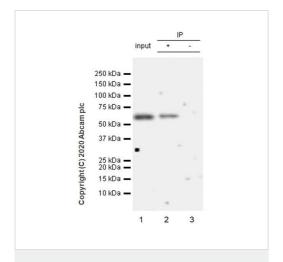


Immunoprecipitation - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647) HEXIM1 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) (treated with 10 μ M MG-132 for 24 hours), whole cell lysate with ab240647 at 1/30 dilution (2ug in 0.35mg lysates). Western blot 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 μM MG-132 for 24 hours, whole cell lysate 10 ug

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

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab240647 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.



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

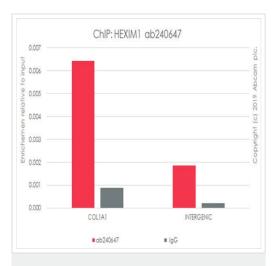
Lane 2: ab240647 IP in MEF whole cell lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) 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 $4\,^\circ\text{C}$.

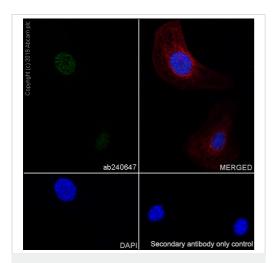


ChIP - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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 μ g of chromatin, 5 μ g of ab 240647 (red), or 5 μ g of rabbit normal lgG <u>ab172730</u> (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). Primers and probes are from paper PMCID: PMC4103662

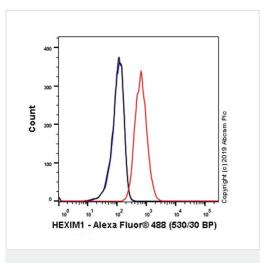
*https://www.abcam.com/resources? keywords=X%20ChIP%20protocol



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).

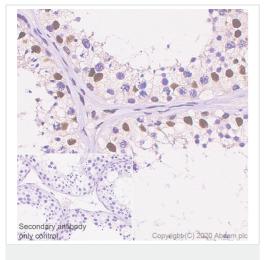
Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



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 (0.1ug) (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, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

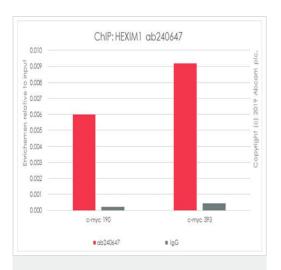


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HEXIM1 antibody
[EPR23430-12] - ChIP Grade (ab240647)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling HEXIM1 with ab240647 at 1/2000 dilution 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.

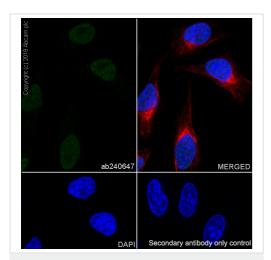


ChIP - Anti-HEXIM1 antibody [EPR23430-12] - ChIP Grade (ab240647)

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 μ g of chromatin, 5 μ g of ab240647 (red), or 5 μ g of rabbit normal lgG <u>ab172730</u> (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).

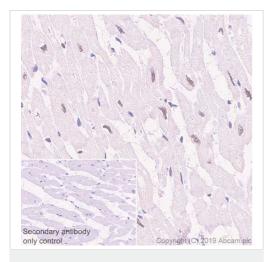
https://www.abcam.com/resources? keywords=X%20ChIP%20protocol



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[®] 488) antibody at 1/1000 (Green). Confocal image showing nuclear staining in HeLa cells. **ab195889** 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 IgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.



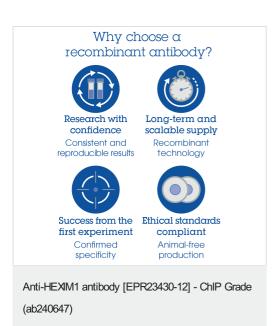
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HEXIM1 antibody

[EPR23430-12] - ChIP Grade (ab240647)

Immunohistochemical analysis of paraffin-embedded Human cardiac muscle tissue labeling HEXIM1 with ab240647 at 1/2000 dilution 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® 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.



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