# abcam

# Product datasheet

# Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade ab175932



重组 RabMAb

★★★★★ 2 Abreviews 57 References 18 图像

概述

产品名称 Anti-NRF1抗体[EPR5554(N)] - ChIP Grade

描述 兔单克隆抗体[EPR5554(N)] to NRF1 - ChIP Grade

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), ChIP, WB, ICC/IF, IP, ChIC/CUT&RUN-seq, IHC-P, ChIP-sequencing

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human NRF1 aa 350-450 (Cysteine residue). The exact sequence is

proprietary.

Database link: Q16656

WB: MCF-7, HeLa and 293T cell lysates and human fetal heart, mouse heart, mouse brain, rat 阳性对照

> heart and rat brain tissue lysates. IHC-P: Human gastric adenocarcinoma, human cervical carcinoma and human skeletal muscle tissues. ICC/IF: HeLa and MCF-7 cells. Flow Cyt (intra):

293T cells. IP: 293T cell lysate. ChIP-Seq: HeLA cells.

常规说明 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**.

性能

形式

存放说明 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.

存储溶液 Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR5554(N)

**同种型** IgG

#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab175932于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/10 - 1/150. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.
WB	<b>★★★★ (2)</b>	1/1000 - 1/10000. Predicted molecular weight: 54 kDa.
ICC/IF		1/50 - 1/100.
IP		1/10 - 1/100.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
ChIP-sequencing		Use 8µg for 10 <sup>7</sup> cells.

功能	Transcription factor that activates the expression of the EIF2S1 (EIF2-alpha) gene. Links the

transcriptional modulation of key metabolic genes to cellular growth and development. Implicated in the control of nuclear genes required for respiration, heme biosynthesis, and mitochondrial

DNA transcription and replication.

组织**特异性** Ubiquitously expressed with strongest expression in skeletal muscle.

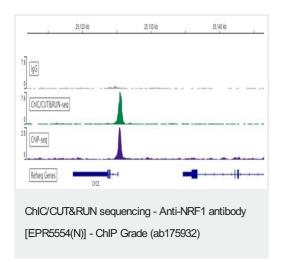
序列相似性 Belongs to the NRF1/Ewg family.

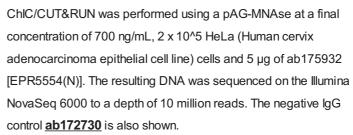
翻译后修饰 Phosphorylation enhances DNA binding.

细胞定位 Nucleus.

### 图片

靶标

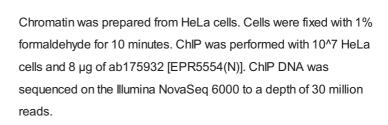




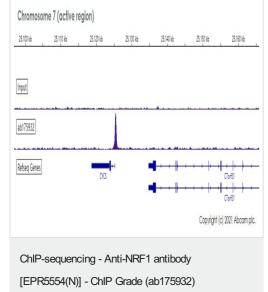
The ChIP data was conducted on chromatin prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 8  $\mu$ g of ab175932. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

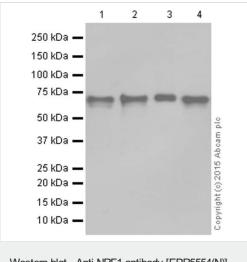
Additional screenshots of mapped reads can be downloaded <u>here</u>.

The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.



Additional screenshots of mapped reads can be downloaded  $\underline{\textbf{here}}.$ 





Western blot - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

**All lanes :** Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932) at 1/5000 dilution (purified)

Lane 1 : Mouse heart tissue lysate
Lane 2 : Mouse brain tissue lysate
Lane 3 : Rat heart tissue lysate

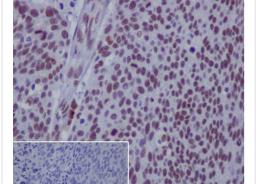
Lysates/proteins at 20 µg per lane.

Lane 4: Rat brain tissue lysate

# **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 54 kDa Observed band size: 68 kDa

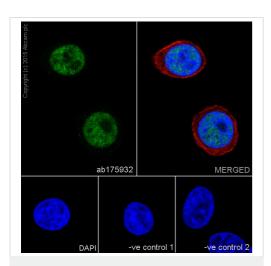


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NRF1 antibody

[EPR5554(N)] - ChIP Grade (ab175932)

Blocking and dilution buffer: 5% NFDM/TBST

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cevical carcinoma tissue labelling NRF1 with purified ab175932 at a dilution of 1/100. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. <a href="mailto:ab97051">ab97051</a>, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

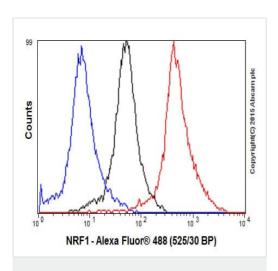


Immunocytochemistry/ Immunofluorescence - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling NRF1 with purified ab175932 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <a href="mailto:ab150077">ab150077</a>, an Alexa Fluor<sup>®</sup> 488-conjugated goat antirabbit lgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. <a href="mailto:ab7291">ab7291</a>, a mouse antitubulin (1/1000) and <a href="mailto:ab150120">ab150120</a>, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse lgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/1000).



Flow Cytometry (Intracellular) - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Intracellular Flow Cytometry analysis of 293T cells labelling NRF1 with purified ab175932 at a dilution of 1/150 (red). Cells were fixed with 80% methanol. A FITC-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. Black - lsotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



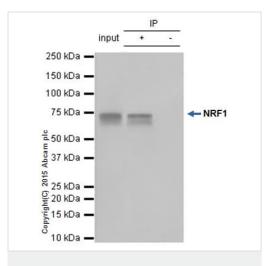
ChIP - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Chromatin was prepared from Hela cells according to the Abcam Dual X-ChIP protocol. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25  $\mu g$  of chromatin, 5  $\mu g$  of ab175932 (red), and 20  $\mu l$  of Protein A/G sepharose beads. 5  $\mu g$  of rabbit normal l g G was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

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



Immunoprecipitation - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

ab175932 (purified) at a dilution of 1/50 immunoprecipitating NRF1 in 293T whole cell lysate.

Lane 1 (input): 293T whole cell lysate (10 $\mu$ g)

Lane 2 (+): ab175932 + 293T whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab175932 in 293T whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



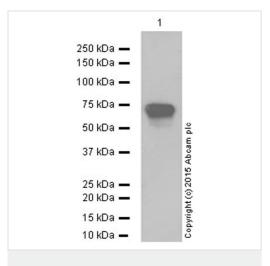
ChIP - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Chromatin was prepared from NIH/3T3 treated with MG-132(2uM 16h) cells according to the Abcam Dual X-ChIP protocol\*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25  $\mu g$  of chromatin, 5  $\mu g$  of ab175932 (red), and 20  $\mu l$  of Protein A/G sepharose beads. 5  $\mu g$  of rabbit normal l g G was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

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



Western blot - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

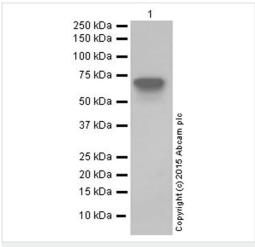
Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932) at 1/10000 dilution (purified) + HEK293 whole cell lysate at 20  $\mu$ g

#### **Secondary**

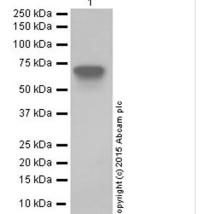
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 68 kDa

Blocking and dilution buffer: 5% NFDM/TBST



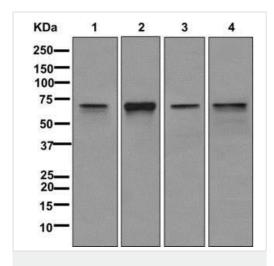
Western blot - Anti-NRF1 antibody [EPR5554(N)] -ChIP Grade (ab175932)



Blocking and dilution buffer: 5% NFDM/TBST

Predicted band size: 54 kDa

Observed band size: 68 kDa



Western blot - Anti-NRF1 antibody [EPR5554(N)] -ChIP Grade (ab175932)

All lanes: Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932) at 1/1000 dilution (unpurified)

Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932) at

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

1/10000 dilution (purified) + HeLa whole cell lysate at 20 µg

Lane 1: MCF-7 cell lysate Lane 2: Hela cell lysate

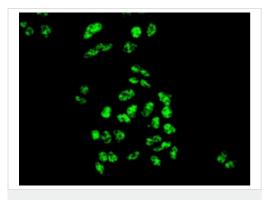
**Secondary** 

Lane 3: Human fetal heart tissue lysate

Lane 4: 293T cell lysate

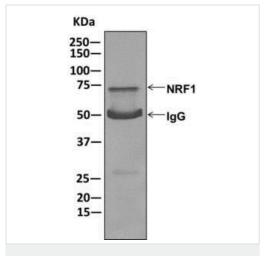
Lysates/proteins at 10 µg per lane.

Predicted band size: 54 kDa



Immunocytochemistry/ Immunofluorescence - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

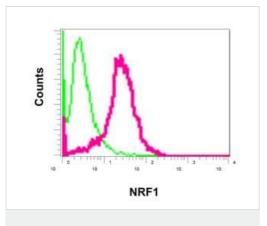
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling NRF1 with unpurified ab175932 at a dilution of 1/50.



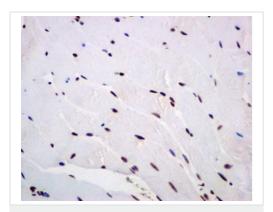
immunoprecipitating NRF1 in 293T cell lysate.

ab175932 (unpurified) at a dilution of 1/10





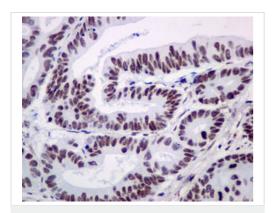
Flow Cytometry (Intracellular) - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932) Intracellular flow cytometric analysis of permeabilized 293T cells labeling NRF1 with unpurified ab175932 at a dilution of 1/10 (red) compared to a negative control (rabbit lgG,green).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue labeling NRF1 with unpurified ab175932 at a dilution of 1/50.

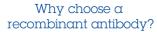
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labeling NRF1 with unpurified ab175932 at a dilution of 1/50.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.





Research with confidence Consistent and



Long-term and scalable supply Recombinant





Success from the Ethical standards first experiment Confirmed specificity

compliant Animal-free production

Anti-NRF1 antibody [EPR5554(N)] - ChIP Grade (ab175932)

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

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