abcam

Product datasheet

Anti-NR2F2 antibody [EPR18443] ab211777



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Overview

Product name Anti-NR2F2 antibody [EPR18443]

Description Rabbit monoclonal [EPR18443] to NR2F2

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, ICC/IF, IP Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HCT116, C6, PC-12, NIH/3T3, MCF7, K562, HEK-293 and HEK-293T whole cell lysates;

> human fetal kidney tissue lysate; rat brain and spleen tissue lysates. IHC-P: Human tonsil, testis, fetal spleen, breast cancer and colon cancer tissue; mouse liver tissue; rat lung tissue. ICC/IF:

NIH/3T3 and HEK-293 cells. IP: MCF7 whole cel lysate.

General notes 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**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clonality Monoclonal Clone number EPR18443

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab211777 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		1/1000. Predicted molecular weight: 46 kDa.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
IP		1/50.

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Function Ligand-activated transcription factor. Activated by high concentrations of 9-cis-retinoic acid and

all-trans-retinoic acid, but not by dexamethasone, cortisol or progesterone (in vitro). Regulation of

the apolipoprotein A-I gene transcription. Binds to DNA site A.

Tissue specificity Ubiquitous.

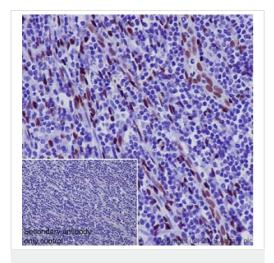
Involvement in diseaseCongenital heart defects, multiple types, 4

Sequence similaritiesBelongs to the nuclear hormone receptor family. NR2 subfamily.

Contains 1 nuclear receptor DNA-binding domain.

Cellular localization Nucleus.

Images



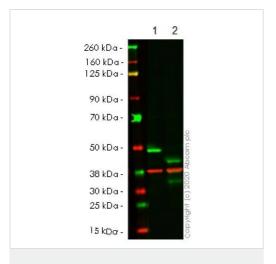
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear staining on mesenchymal cells of human tonsil tissue (PMID: 11026559) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-NR2F2 antibody [EPR18443] (ab211777)

Lane 1: Anti-TRIM24 antibody [EPR22825-2] (<u>ab256491</u>) at 1/1000 dilution

Lane 2: Anti-NR2F2 antibody [EPR18443] (ab211777) at 1/1000 dilution

Lane 1: Wild-type HCT116 cell lysate

Lane 2: NR2F2 CRISPR/Cas9 edited HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 46 kDa Observed band size: 45 kDa

Lanes 1-2: Merged signal (red and green). Green - ab211777 observed at 45 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab211777 was shown to react with NR2F2 in wild-type HCT116 cells in western blot. The band observed in CRISPR/Cas9 edited cell line ab266888 (CRISPR/Cas9 edited cell lysate ab257186) lane below 45kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HCT116 and NR2F2 CRISPR/Cas9 edited HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk.

ab211777 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

250 kDa -250 kDa -250 kDa -250 kDa -150 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -100 kDa -100 kDa -75 kDa -75 kDa -75 kDa -75 kDa -50 kDa -50 kDa = 50 kDa -50 kDa -37 kDa -37 kDa 37 kDa -37 kDa -25 kDa -25 kDa -25 kDa -25 kDa -20 kDa -20 kDa -20 kDa -20 kDa -15 kDa -15 kDa -15 kDa -15 kDa -10 kDa -10 kDa -10 kDa -10 kDa -

Western blot - Anti-NR2F2 antibody [EPR18443] (ab211777)

All lanes : Anti-NR2F2 antibody [EPR18443] (ab211777) at 1/1000 dilution

Lane 1 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate

Lane 2: HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 3: Human fetal kidney tissue lysate

Lane 4: HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lane 5 : K562 (human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

Lanes 1-2 & 4-5 : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

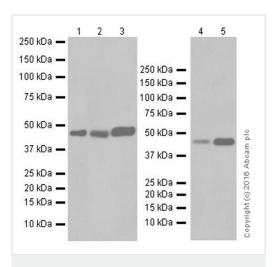
Lane 3: Goat Anti-Rabbit lgG Peroxidase Conjugate, specific to the non-reduced form of lgG at 1/10000 dilution

Developed using the ECL technique.

Predicted band size: 46 kDa
Observed band size: 46 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM /TBST



Western blot - Anti-NR2F2 antibody [EPR18443] (ab211777)

All lanes : Anti-NR2F2 antibody [EPR18443] (ab211777) at 1/1000 dilution

Lane 1: C6 (rat glial tumor cell line) whole cell lysate

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

Lane 3: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 4 : Rat brain tissue lysate
Lane 5 : Rat spleen tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

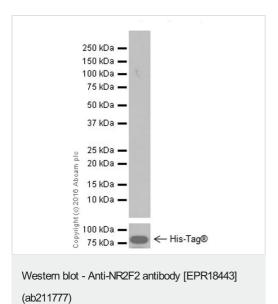
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 46 kDa **Observed band size:** 46 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM /TBST



Anti-NR2F2 antibody [EPR18443] (ab211777) at 1/1000 dilution + Human NR2F1 full length recombinant protein (negative control) at $10~\mu g$

Secondary

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

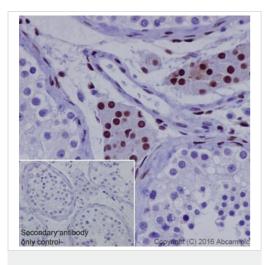
Developed using the ECL technique.

Predicted band size: 46 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST

Human NR2F1 full length recombinant protein containing aa1-423 with a His-tag was made in house.

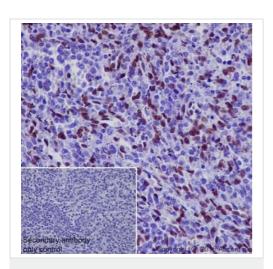


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mesenchymal cells of human testis tissue (PMID: 11026559) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

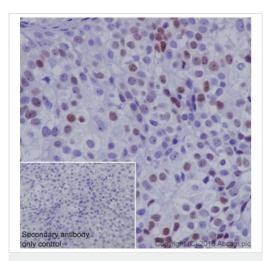


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded human fetal spleen tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mesenchymal cells of human fetal spleen tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

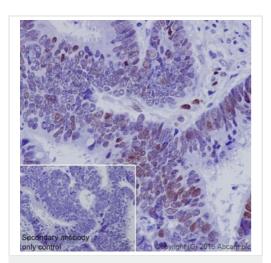


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on tumor cells of human breast cancer tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

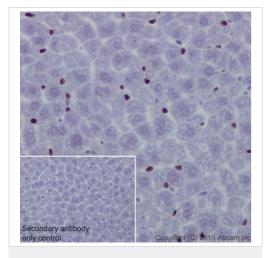


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on tumor cells of human colon cancer tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

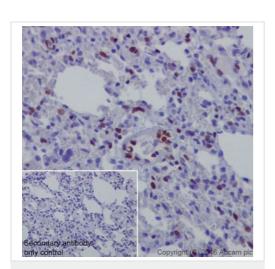


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mesenchymal cells of mouse liver tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

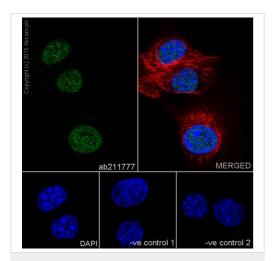


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NR2F2 antibody
[EPR18443] (ab211777)

Immunohistochemical analysis of paraffin-embedded rat lung tissue labeling NR2F2 with ab211777 at 1/250 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mesenchymal cells of rat lung tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



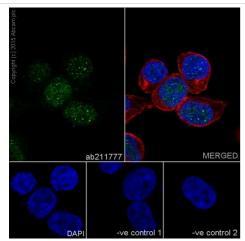
Immunocytochemistry/ Immunofluorescence - Anti-NR2F2 antibody [EPR18443] (ab211777)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling NR2F2 with ab211777 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line.

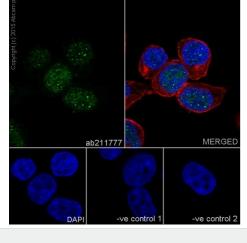
The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

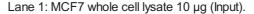
- -ve control 1: ab211777 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) preadsorbed (<u>ab150120</u>) secondary at 1/1000 dilution.
- -ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-NR2F2 antibody [EPR18443] (ab211777)



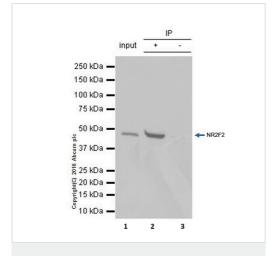
NR2F2 was immunoprecipitated from 1 mg of MCF7 (human breast adenocarcinoma cell line) whole cell lysate with ab211777 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab211777 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.



Lane 2: ab211777 IP in MCF7 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab211777 in MCF7 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 1 second.



Immunoprecipitation - Anti-NR2F2 antibody [EPR18443] (ab211777)

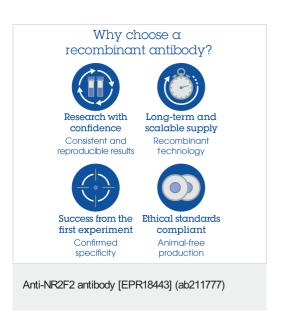
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293 (human epithelial cell line from embryonic kidney) cells labeling NR2F2 with ab211777 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HEK293 cell line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:

-ve control 1: ab211777 at 1/100 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) secondary at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) secondary at 1/1000 dilution.



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