abcam

ab214549

Anti-Nanog antibody [EPR20694] - ChIP Grade

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20694
Purification technique	Affinity purification Protein A
Concentration	0.609 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Mouse
Dilution info	5 µg
Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

ChIP

Tested

Species	Mouse
Dilution info	5 µg for 25 µg chromatin
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	1/60
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Target data

See full target information Nanog

Function

Transcription regulator involved in inner cell mass and embryonic stem (ES) cells proliferation and self-renewal (PubMed:25825768). Imposes pluripotency on ES cells and prevents their differentiation towards extraembryonic endoderm and trophectoderm lineages. Blocks bone morphogenetic protein-induced mesoderm differentiation of ES cells by physically interacting with SMAD1 and interfering with the recruitment of coactivators to the active SMAD transcriptional complexes. Acts as a transcriptional activator or repressor. Binds optimally to the DNA consensus sequence 5'-TAAT[GT][GT]-3' or 5'-[CG][GA][CG]C[GC]ATTAN[GC]-3'. Binds to the POU5F1/OCT4 promoter (By similarity). Able to autorepress its expression in differentiating (ES) cells: binds to its own promoter following interaction with ZNF281/ZFP281, leading to recruitment of the NuRD complex and subsequent repression of expression. When overexpressed, promotes cells to enter into S phase and proliferation.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Nanog also known as Nanog homeobox is a transcription factor playing an important role in maintaining the pluripotency of embryonic stem cells. The molecular weight of Nanog is approximately 35 kDa. It is found mainly in the inner cell mass of the blastocyst but it also expresses in embryonic stem cells and some adult stem cell populations. Nanog acts by binding to DNA in a sequence-specific manner to regulate gene expression essential for stem cell self-renewal and pluripotency.
Biological function summary	Nanog plays a central role in stem cell biology operating as part of a complex regulatory network. Nanog interacts with other important transcription factors like Oct4 and Sox2 forming a core pluripotency network. This network suppresses the differentiation of stem cells by regulating the expression of genes involved in cell fate decisions. By modulating the expression of different pathways Nanog ensures the maintenance of an undifferentiated state in stem cells.
Pathways	Nanog is deeply embedded in the Wnt/β-Catenin and TGF-β signaling pathways important for stem cell maintenance and differentiation. In the Wnt/β-Catenin pathway Nanog works alongside proteins like β-catenin to drive the expression of genes that promote self-renewal. Meanwhile in the TGF-β pathway Nanog acts with proteins such as Smad2/3 to balance pluripotency and differentiation signals. These pathways critically support the complex network that sustains stem cell identity and function.
Associated diseases and disorders	Nanog's expression relates closely to its role in various cancers such as glioblastoma and colorectal cancer. Abnormally high levels of Nanog contribute to the tumorigenicity of cancer cells by maintaining their self-renewal and undifferentiated state similar to its role in stem cells. Nanog interacts with proteins like p53 known for its tumor suppressor functions often leading to challenges in cancer treatment. Understanding Nanog's influence in these pathways provides valuable insights into therapeutic targets for combating cancer stem cell resilience.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

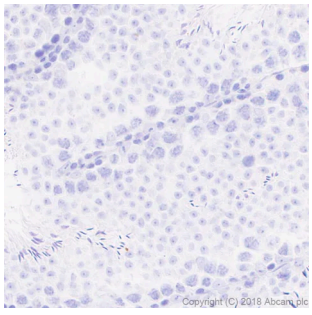
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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9 product images

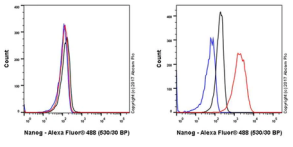


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Nanog antibody

Immunohistochemical analysis of paraffin-embedded adult mouse testis tissue labeling Nanog with ab214549 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. **Negative tissue:** No staining on adult mouse testis is observed(PMID: 12787505; PMID: 15939376). Counter stained with Hematoxylin.

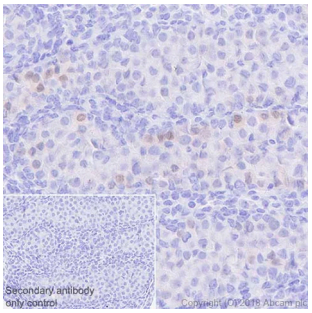
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog Flow Cytometry (Intracellular) staining using rabbit Anti-Nanog antibody

Intracellular flow cytometric analysis of 4 % paraformaldehyde-fixed, 90% methanol permeabilized F9 (mouse embryonic testicular cancer cell line, Right) and NIH/3T3 (mouse embryo fibroblast cell line, Left) cells labeling Nanog with ab214549 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.
Negative control: NIH/3T3 cell line.

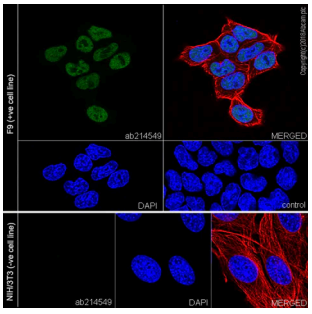


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Nanog antibody

Immunohistochemical analysis of paraffin-embedded mouse E14.5 testis tissue labeling Nanog with ab214549 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Mainly nuclear staining is observed in testis of mouse embryo E14.5 (PMID: 15939376; PMID: 12787505). Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

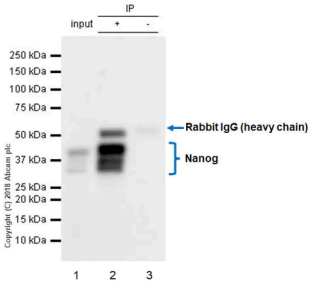


Immunocytochemistry/ Immunofluorescence - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Nanog antibody

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeablized F9 (mouse embryonic testicular cancer cell line) and NIH/3T3 (mouse embryo fibroblast cell line) cells labeling Nanog with ab214549 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 in F9 cell line. **Negative control:** NIH/3T3 (PMID: 17352742).
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog was immunoprecipitated from 0.35 mg of F9 (mouse embryonic testicular cancer cell line) whole cell lysate with ab214549 at 1/1000 dilution. Western blot was performed from the immunoprecipitate using ab214549 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5,000 dilution
Lane 1: F9 whole cell lysate 10 µg (input)

Lane 2: ab214549 IP in F9 whole cell lysate.

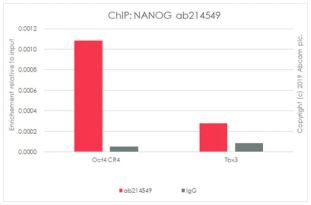
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab214549 in F9 whole cell lysate.

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

Exposure time: 15 seconds.

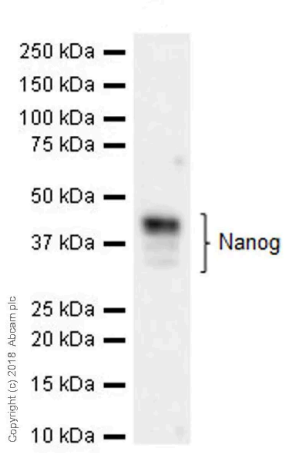
The multiple bands observed are consistent with the literature (PMID: 24936455).
All lanes:
Immunoprecipitation - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Predicted band size: 34 kDa



ChIP - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Chromatin was prepared from F9 cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5µg of ab214549 (red), and 20 µl of protein A/G sepharose beads slurry (10 µl of sepharose A beads + 10 µl of sepharose G beads). Then 5 µg of rabbit normal IgG was added to the control beads (grey). The immunoprecipitated DNA was quantified by real time PCR (SYBR green chemistry).



Western blot - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Blocking/Dilution buffer: 5% NFDM/TBST.
The multiple bands observed are consistent with the literature (PMID: 24936455).

All lanes:
Western blot - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549) at 1/1000 dilution

All lanes:
ES-D3 (mouse embryonic multipotent stem cell line) whole cell lysate at 10 µg

Secondary

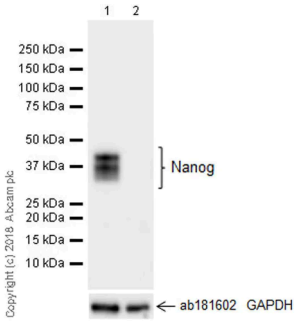
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 34 kDa

Observed band size: 29-42 kDa

Exposure time: 3min



Western blot - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Nanog Western blot staining using rabbit Anti-Nanog antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
The multiple bands observed are consistent with the literature (PMID: 24936455). Negative control: NIH/3T3 (PMID: 12787505).

All lanes:
Western blot - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549) at 1/1000 dilution

Lane 1:
F9 (Mouse embryonic testicular cancer cell line) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate at 20 µg

Secondary

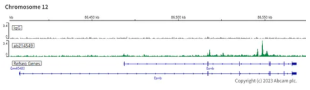
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 34 kDa

Observed band size: 29-42 kDa

Exposure time: 70s



ChIC/CUT&RUN sequencing - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 F9 (Mouse embryonic testicular cancer cell line) cells and 5µg of ab214549 [EPR20694]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.