

Product datasheet

Anti-Nanog antibody [EPR20694] - ChIP Grade ab214549

Recombinant RabMAb

★★★★★ [3 Abreviews](#) [5 References](#) [10 Images](#)

Overview

Product name	Anti-Nanog antibody [EPR20694] - ChIP Grade
Description	Rabbit monoclonal [EPR20694] to Nanog - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: ChIC/CUT&RUN-seq, Flow Cyt (Intra), ChIP, IHC-P, WB, ICC/IF, IP
Species reactivity	Reacts with: Mouse
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: F9 and ES-D3 whole cell lysates. IHC-P: Mouse E14.5 testis tissue. ICC/IF: F9 cells. Flow Cyt (intra): F9 cells. IP: F9 whole cell lysate. ChIP: Chromatin prepared from F9 cells. ChIC/CUT&RUN-Seq: F9 cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR20694
 Isotype IgG

Applications

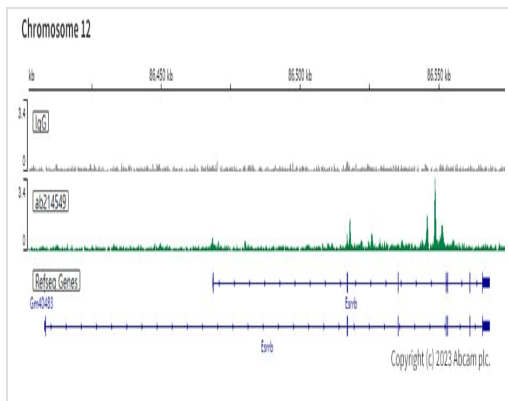
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab214549 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
Flow Cyt (Intra)		1/60.
ChIP		Use 5 µg for 25 µg of chromatin.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 29-42 kDa (predicted molecular weight: 34 kDa).
ICC/IF	★★★★★ (1)	1/100.
IP		1/30.

Target

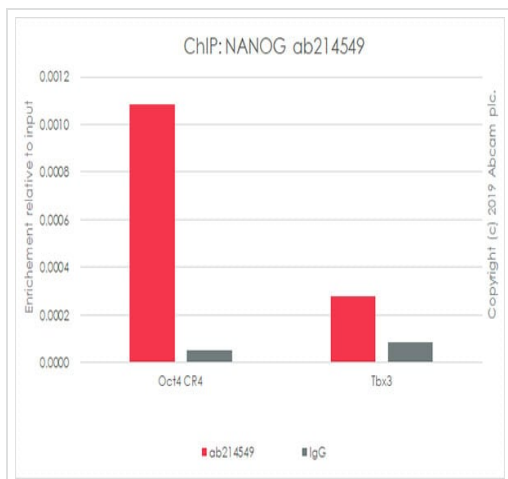
Function	Transcription regulator involved in inner cell mass and embryonic stem (ES) cells proliferation and self-renewal. Imposes pluripotency on ES cells and prevents their differentiation towards extraembryonic endoderm and trophoctoderm lineages. Blocks bone morphogenetic protein-induced mesoderm differentiation of ES cells by physically interacting with SMAD1 and interfering with the recruitment of coactivators to the active SMAD transcriptional complexes (By similarity). Acts as a transcriptional activator or repressor (By similarity). Binds optimally to the DNA consensus sequence 5'-TAAT[GT][GT]-3' or 5'-[CG][GA][CG]C[GC]ATTAN[GC]-3' (By similarity). When overexpressed, promotes cells to enter into S phase and proliferation.
Tissue specificity	Expressed in testicular carcinoma and derived germ cell tumors (at protein level). Expressed in fetal gonads, ovary and testis. Also expressed in ovary teratocarcinoma cell line and testicular embryonic carcinoma. Not expressed in many somatic organs and oocytes.
Sequence similarities	Belongs to the Nanog homeobox family. Contains 1 homeobox DNA-binding domain.
Developmental stage	Expressed in embryonic stem (ES) and carcinoma (EC) cells. Expressed in inner cell mass (ICM) of the blastocyst and gonocytes between 14 and 19 weeks of gestation (at protein level). Not expressed in oocytes, unfertilized oocytes, 2-16 cell embryos and early morula (at protein level). Expressed in embryonic stem cells (ES). Expression decreases with ES differentiation.
Cellular localization	Nucleus.



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

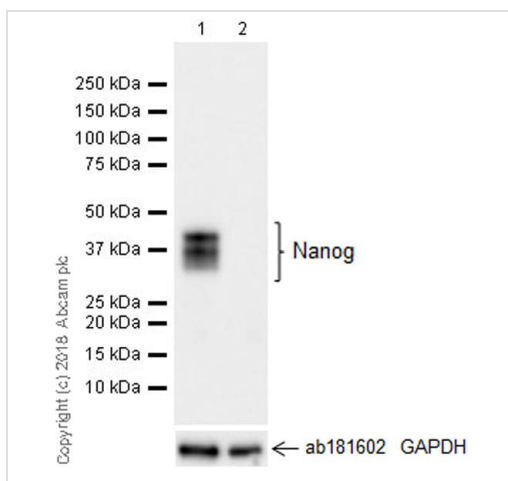
ChIP/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10⁵ F9 (Mouse embryonic testicular cancer cell line) cells and 5µg of ab214549 [EPR20694]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded [here](#). The University of Geneva owns patents relevant to ChIP (Chromatin Immuno-Cleavage) methods.



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)

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

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

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

Lysates/proteins at 20 µg per lane.

Secondary

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

Developed using the ECL technique.

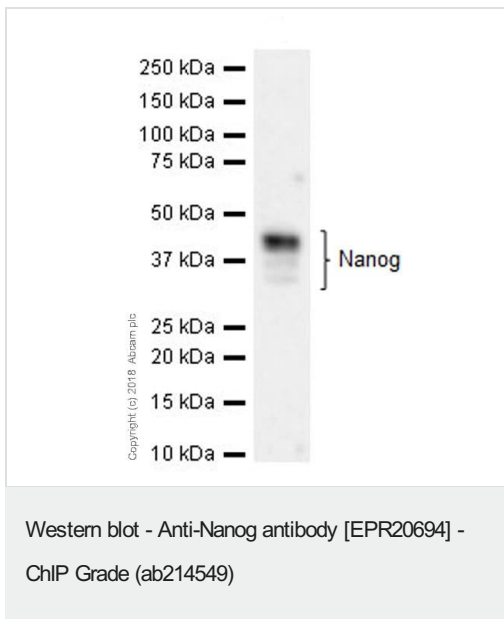
Predicted band size: 34 kDa

Observed band size: 29-42 kDa

Exposure time: 70 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The multiple bands observed are consistent with the literature (PMID: 24936455). Negative control: NIH/3T3 (PMID: 12787505).



Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549) at 1/1000 dilution + ES-D3 (mouse embryonic multipotent stem cell line) whole cell lysate at 10 μ g

Secondary

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

Developed using the ECL technique.

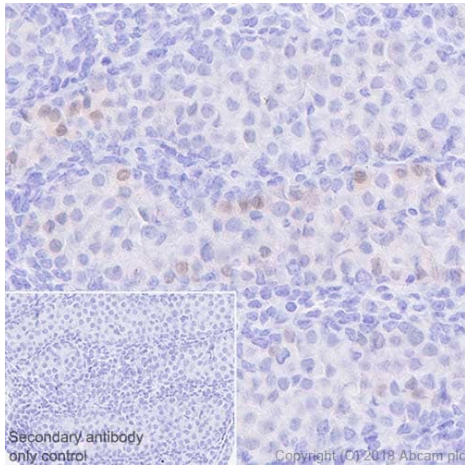
Predicted band size: 34 kDa

Observed band size: 29-42 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The multiple bands observed are consistent with the literature (PMID: 24936455).

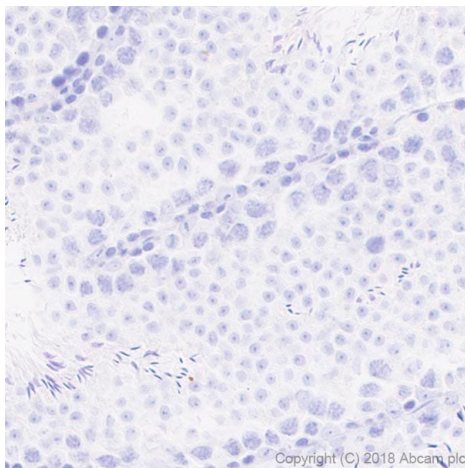


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

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.



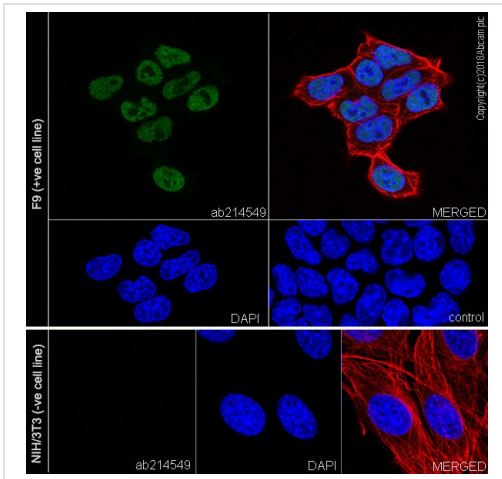
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

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.

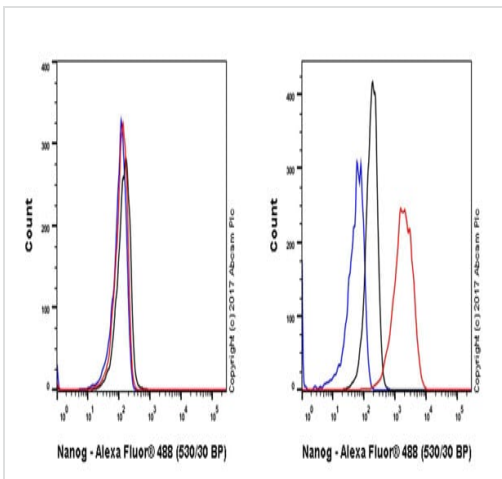


Immunocytochemistry/ Immunofluorescence - Anti-Nanog antibody [EPR20694] - ChIP Grade (ab214549)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 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.

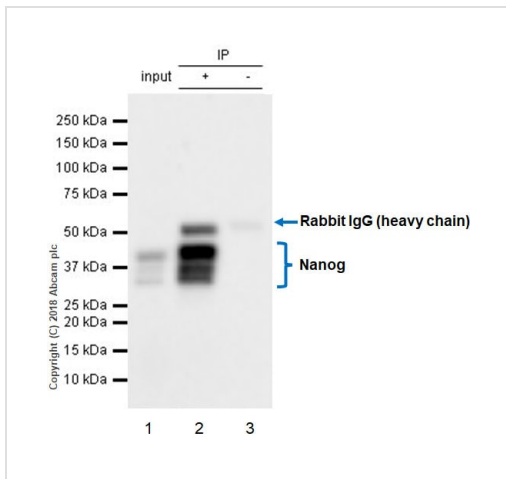
Control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



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

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.



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).

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

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