abcam

Product datasheet

Anti-Nanog antibody [EPR2027(2)] ab109250



*** 11 Abreviews 154 References 15 Images

Overview

Product name Anti-Nanog antibody [EPR2027(2)]

Description Rabbit monoclonal [EPR2027(2)] to Nanog

Host species Rabbit

Specificity 100% identities with NANOGP8

Tested applications Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra), ChIP-sequencing, IP

Species reactivity Reacts with: Human

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

Positive control WB: NCCIT cell lysate. IHC-P: Human seminoma tissue, Human dysgerminoma tissue and

Human embryonal carcinoma tissue. ICC/IF: Human embryonic carcinoma and Human liver cell

lines. IP: NCCIT whole cell lysate. ChIP-seq: NCCIT cells.

Please note that Nanog is expressed variably in different tissues and that optimisation may be **General notes**

required depending on the tissue used for the experiment.

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**.

Mouse: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

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: 7.20

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clonality Monoclonal
Clone number EPR2027(2)

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab109250 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	****(4)	1/1000 - 1/10000. Detects a band of approximately 37 kDa (predicted molecular weight: 35 kDa).
IHC-P	★★★☆☆ (3)	1/100 - 1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. Antigen retrieval is recommended.
ICC/IF	★★★★ (4)	1/100 - 1/250.
Flow Cyt (Intra)		1/70. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ChIP-sequencing		Use at an assay dependent concentration.
IP		1/40.

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 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 (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.

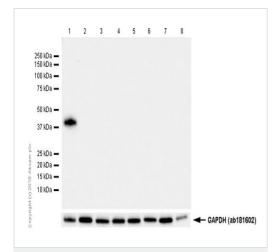
Developmental stage

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.

Images



Western blot - Anti-Nanog antibody [EPR2027(2)] (ab109250)

All lanes : Anti-Nanog antibody [EPR2027(2)] (ab109250) at 1/2000 dilution

Lane 1 : NCCIT (Human pluripotent embryonic carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 3: HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 4: MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 5: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 6 : Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 7: HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lane 8 : PANC-1 (Human pancreatic epithelioid carcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST

Lysates/proteins at 20 µg per lane.

Secondary

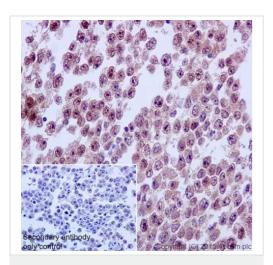
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 35 kDa **Observed band size:** 37 kDa

Exposure time: 30 seconds

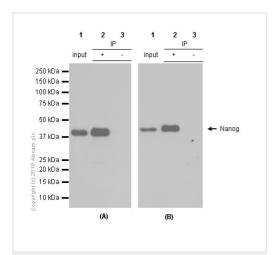
Nanog is highly expressed in cancer stem cells. Although some papers support the expression in undifferentiated cancer cell lines, such as HeLa (PMID: 22337995, 28092370), MDA-MB-231 (PMID:

28401007, 25919570), HepG2 (PMID: 29477378), Huh7 (PMID: 26919045), HCT 116 (PMID: 25249558, 28092370) and PANC-1 (PMID: 28703793, 25846752), ab109250 can't detect the target band in these cell lines, even at the dilution of 1:200.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human seminoma tissue labelling Nanog with purified ab109250 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a goat antirabbit lgG H&L (HRP) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunoprecipitation - Anti-Nanog antibody [EPR2027(2)] (ab109250)

ab109250 (purified) at 1/40 dilution (1.5 μ g/ml) immunoprecipitating Nanog in NCCIT whole cell lysate.

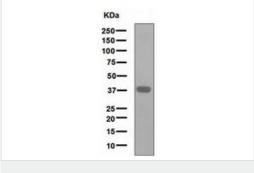
Lane 1 (input): NCCIT(Human pluripotent embryonic carcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab109250 & NCCIT whole cell lysate

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab109250 in NCCIT whole cell lysate

For western blotting, ab109250 at 1/500 dilution (1.5 μ g/ml) VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/1000 dilution.

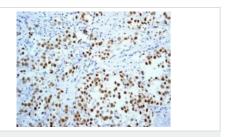
Blocking and diluting buffer: 5% NFDM /TBST.



Western blot - Anti-Nanog antibody [EPR2027(2)] (ab109250)

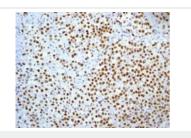
Anti-Nanog antibody [EPR2027(2)] (ab109250) at 1/1000 dilution (unpurified) + NCCIT cell lysate at 10 μg

Predicted band size: 35 kDa **Observed band size:** 37 kDa



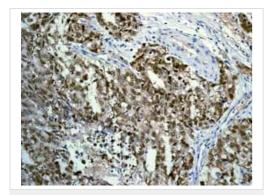
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human seminoma tissue labelling Nanog with unpurified ab109250 at 1/100.



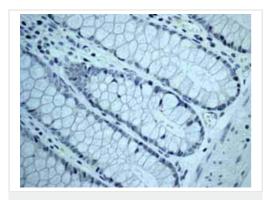
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human dysgerminoma tissue labelling Nanog with unpurified ab109250 at 1/100.



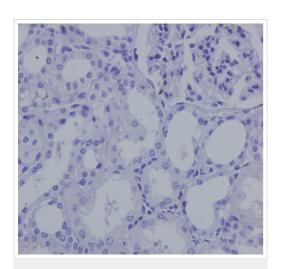
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human embryonal carcinoma tissue labelling Nanog with unpurified ab109250 at 1/100.



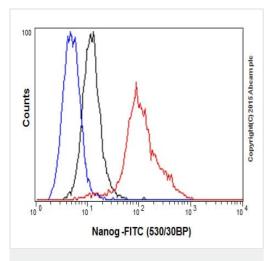
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human normal colon tissue shows negative staining of Nanog with unpurified ab109250.



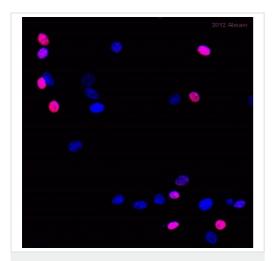
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nanog antibody
[EPR2027(2)] (ab109250)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human adult kidney tissue shows negative staining of Nanog with unpurified ab109250.



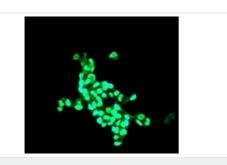
Flow Cytometry (Intracellular) - Anti-Nanog antibody [EPR2027(2)] (ab109250)

Intracellular Flow Cytometry analysis of NCCIT cells labelling Nanog with purified ab109250 at 1/70 (red). Cells were fixed with 4% paraformal dehyde. 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.



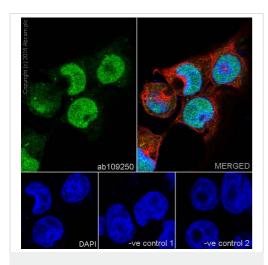
Immunocytochemistry/ Immunofluorescence - Anti-Nanog antibody [EPR2027(2)] (ab109250) This image is courtesy of an anonymous Abreview

Immunocytochemistry/Immunofluorescence analysis of Human Liver cells labelling Nanog with unpurified ab109250. Cells were fixed with Paraformaldehyde, permeabilized with Triton X-100 0.1% and blocked with 1% BSA for 12 hours at 4°C. Sample was incubated with primary antibody (1/500 in PBS) for 16 hour at 4°C. An Alexa Fluor[®]647-conjugated Donkey anti-rabbit(1/1000) lgG polyclonal was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Nanog antibody [EPR2027(2)] (ab109250)

Immunocytochemistry/Immunofluorescence analysis of embryonic carcinoma cells labelling Nanog with unpurified ab109250 at 1/100.



Immunocytochemistry/ Immunofluorescence - Anti-Nanog antibody [EPR2027(2)] (ab109250)

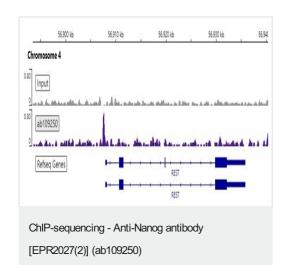
Immunocytochemistry/Immunofluorescence analysis of NCCIT(human pluripotent embryonal carcinoma) cells labelling Nanog with purified ab109250 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/500).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).

Chromatin was prepared from NCCIT (Human pluripotent embryonic carcinoma cell line) cells. ChIP was performed with 10^7 NCCIT cells and 8 μ g of ab109250 [[EPR2027(2)]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded here.





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