Anti-Nanog Antibody [SC05-70] - BSA and Azide free

HA750204

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Tissue, FC, IHC-P, ChIP
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	SC05-70

Description: Nanog (from “Tir Na Nog,” the mythologic Celtic land of the ever young) is a divergent homeodomain protein that directs pluripotency and differentiation of undifferentiated embryonic stem cells. Nanog mRNA is present in pluripotent mouse and human cell lines and absent from differentiated cells. Human Nanog protein shares 52% overall amino acid identity with the mouse protein and 85% identity in the homeodomain. Human Nanog maps to gene locus 12p13.31, whereas mouse Nanog maps to gene loci 6 F2. Murine embryonic Nanog expression is detected in the inner cell mass of the blastocyst. High levels of human Nanog expression have been detected by Northern analysis in the undifferentiated NTERA-2 cl.D1 embryonal carcinoma cell line.

Immunogen: Recombinant protein within Human Nanog aa 9-49 / 305.

Positive control: NCCIT cell lysate, F9, mouse testis tissue, NCCIT, human seminoma tissue.

Subcellular location: Nucleus.

Database links: SwissProt: Q9H9S0 Human | Q80Z64 Mouse

Recommended Dilutions:

WB	1:2,000
IF-Tissue	1:50-1:200
IHC-P	1:50-1:800
FC	1:500-1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

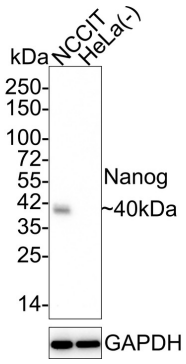

Fig1: Western blot analysis of Nanog on different lysates with Rabbit anti-Nanog antibody (HA750204) at 1/2,000 dilution.

Lane 1: NCCIT cell lysate
Lane 2: HeLa cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 40 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750204) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

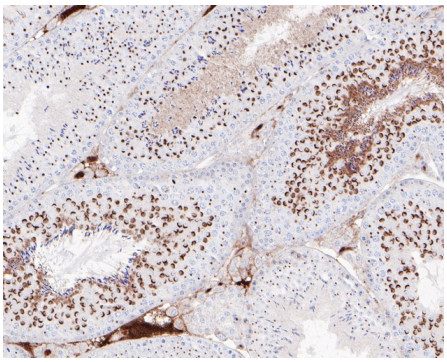

Fig2: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-Nanog antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750204, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

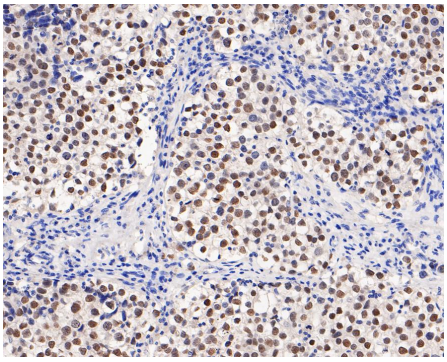

Fig3: Immunohistochemical analysis of paraffin-embedded human seminoma tissue with Rabbit anti-Nanog antibody (HA750204) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750204) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

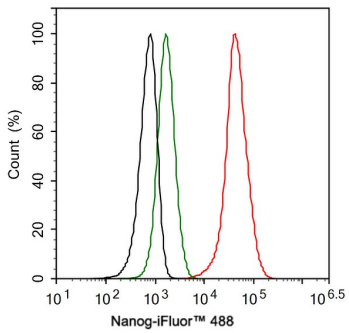

Fig4: Flow cytometric analysis of NCCIT cells labeling Nanog.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750204, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

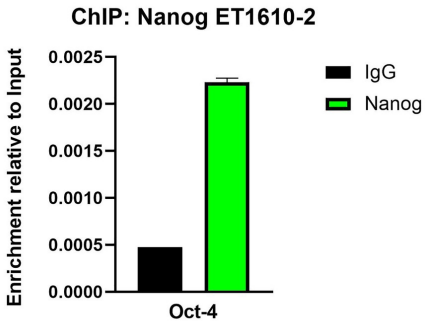

Fig5: Chromatin immunoprecipitations were performed with cross-linked chromatin from NCCIT cells with Nanog (HA750204) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Yang Y et al. Heightened potency of human pluripotent stem cell lines created by transient BMP4 exposure. Proc Natl Acad Sci U S A 112:E2337-46 (2015).
2. Lee K et al. Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated RAG2 and accompanying severe combined immunodeficiency. Proc Natl Acad Sci U S A 111:7260-5 (2014).