Technical Data Sheet

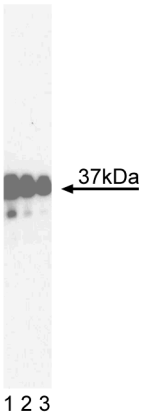
Purified Mouse anti-Human Nanog

Product Information

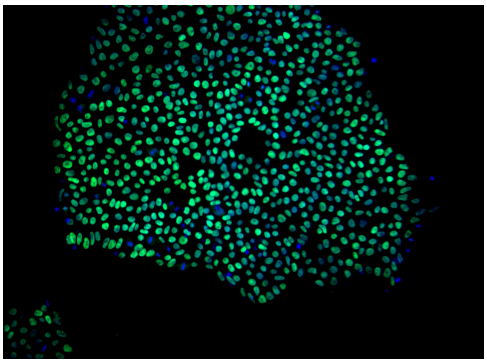
Material Number:	**560482**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	N31-355
Immunogen:	Human Nanog Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed: Human
Target MW:	36-37 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The N31-355 monoclonal antibody reacts with human Nanog (named for Tir Na Nog, the land of the ever-young of Celtic mythology), which is a homeobox transcription factor required for the maintenance of the undifferentiated state of pluripotent stem cells. Nanog expression counteracts the differentiation-promoting signals induced by the extrinsic factors LIF (Leukemia Inhibitory Factor) and BMP (Bone Morphogenic Protein). When Nanog expression is down-regulated, cell differentiation can proceed. Proteins that regulate Nanog expression include transcription factors Oct4, SOX2, FoxD3, and Tcf3 and tumor suppressor p53. Nanog is one of the factors that can contribute to reprogramming of differentiated cells to an induced pluripotent stem cell state.



Western Blot analysis of Nanog in human embryonic stem cells. *Lysate from H9 human ES cells* (WiCell, Madison, WI) was probed with Purified Mouse anti-Human Nanog monoclonal antibody at titrations of 2.0 (lane 1), 1.0 (lane 2), and 0.5 µg/ml (lane 3). Nanog is identified as a band of 36-37 kDa.*
**The H9 cells were cultured on a mitomycin C-treated mouse embryonic fibroblast feeder layer [MEF (CF-1), ATCC SCRC-1040] that maintains the undifferentiated state of the ES cells. The lysate was made from a mixture of the 2 cell types, the majority of which were H9 cells.*



Immunoflourescent staining of Nanog in human embryonic stem cells. *H9 human ES cells (WiCell Madison, WI) passage 31 grown in mTESR™1 media (StemCell Technologies) on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) were fixed, permeabilized, and stained with Purified Mouse anti-Human Nanog monoclonal antibody (pseudo colored green) at 1.2 ug/mL. The second step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Life Technologies) and counter-staining was with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software. Permeabilization using 1x BD Perm/Wash™ Buffer (Cat No. 554723) was used for this antibody; Triton™ X-100 or ice-cold methanol is also suitable for permeabilization.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

BD Biosciences

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging:

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
    a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
    b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
    c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Triton is a trademark of the Dow Chemical Company.
4. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

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