Technical Data Sheet

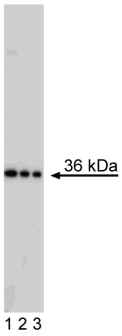
Purified Mouse anti-Human Nanog

Product Information

Material Number:	**560109**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	L96-549
Immunogen:	Human Nanog Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Confirmed: Human
Target MW:	36-37 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

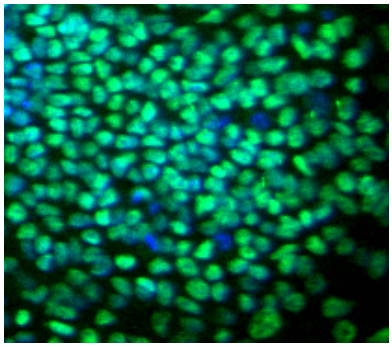
Description

The L96-549 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 (*L*eukemia *I*nhibitory *F*actor) and BMP (*B*one *M*orphogenic *P*rotein). 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.



Western Blot analysis of Nanog in human embryonic stem cell line. *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.*

Western Blot validation of Nanog by RNAi in human embryonal carcinoma cell line. *Lysates from NTERA-2 cl.D1 cells (ATCC CRL-1973, lane 1) and Nanog RNAi-transfected NTERA-2 cl.D1 cells (lane 2) were probed with Purified Mouse anti-Human Nanog monoclonal antibody at 0.5 µg/ml. Down-regulation of Nanog expression is evident in the RNAi-transfected cells (upper blot). Purified Mouse anti-Actin monoclonal antibody (Cat. No. 612656 or 612657) was the gel-loading control (lower blot).*



Immunofluorescent staining of human ES cell line. *The H9 cell line was cultured, fixed, permeabilized, and stained with Purified Mouse anti-Human Nanog monoclonal antibody (pseudo-colored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 647 goat anti-mouse Ig (Invitrogen) and counter-staining was with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer using a 10X objective and merged using BD Attovision™ software. Saponin permeabilization is recommended for this antibody, but not Triton™ X-100 or cold methanol.*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

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, wash the wells twice with 100 µl of 1× PBS, and 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).
3. Remove the fixative from the wells, and wash the wells twice with 100 µl of 1× PBS.
4. Permeabilize the cells by adding 100 µl of 1× BD Perm/Wash™ buffer (Cat. No. 554723) to each well and incubating for 30 minutes at RT.
5. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
6. Dilute the antibody in BD Perm/Wash™ buffer, and stain the cells by adding 50 µl of the diluted antibody to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, dilute the second-step reagent in BD Perm/Wash™ buffer, and stain the cells by adding 50 µl of the diluted second-step reagent to each well and incubating for 1 hour at RT.
9. Remove the diluted second-step reagent, and wash the wells twice with 100 µl of 1× PBS.
10. Remove the PBS, and 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.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

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	NA	(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. Triton is a trademark of the Dow Chemical Company.
3. 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.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.

References

Chambers I. The molecular basis of pluripotency in mouse embryonic stem cells. *Cloning Stem Cells.* 2004; 6(4):386-391. (Biology)
Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell.* 2003; 113:643-655. (Biology)
Ezeh UI, Turek PJ, Reijo RA, Clark AT. Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. *Cancer.* 2005; 104(10):2255-2265. (Biology)
Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell.* 2003; 113:631-642. (Biology)
Pan G, Thomson JA. Nanog and transcriptional networks in embryonic stem cell pluripotency. *Cell Res.* 2007; 17:42-49. (Biology)
Sun Y, Li H, Yang H, Rao MS, Zhan M. Mechanisms controlling embryonic stem cell self-renewal and differentiation. *Crit Rev Eukaryot Gene Expr..* 2006; 16(3):211-231. (Biology)
Suzuki A, Raya A, Kawakami Y, et al. Nanog binds to Smad1 and blocks bone morphogenetic protein-induced differentiation of embryonic stem cells. *Proc Natl Acad Sci U S A.* 2006; 103(27):10294-10299. (Biology)