Technical Data Sheet

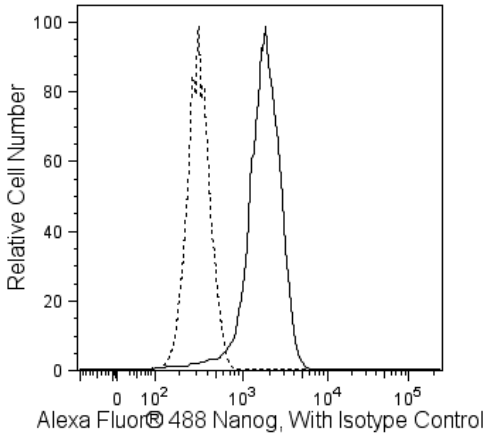
Alexa Fluor® 488 Mouse anti-Human Nanog

Product Information

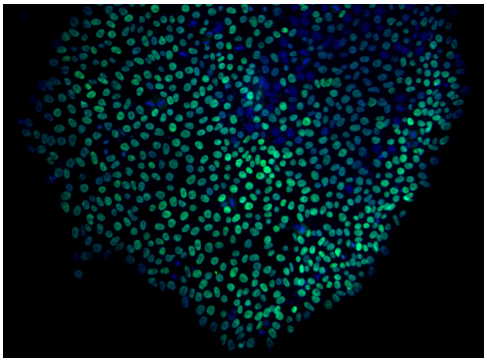
Material Number:	**560791**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	N31-355
Immunogen:	Human Nanog Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	Confirmed: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

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.



Alexa Fluor® 488 anti-human Nanog staining of human embryonic stem cells. *H9 cells (WiCell, Madison, WI), passage 34, were harvested, fixed in BD Cytofix™ buffer (Cat. No. 554655), permeabilized with BD ™ Phosflow Perm/Wash buffer I (Cat. No. 557885) and stained with Alexa Fluor® 488 anti-human Nanog (solid line) or Alexa Fluor® 488 Mouse IgG1, κ Isotype Control (Cat. No.557702, dashed line). Flow cytometry was performed on a BD™ LSR II flow cytometry system. This reagent will also work in BD™ Phosflow Perm II and III buffers.*



Immunoflourescent staining of human embryonic stem cells with Alexa Fluor® 488 anti-human Nanog. *H9 cells (WiCell, Madison, WI), passage 45, grown in mTESR™1 media (StemCell Technologies) on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) were fixed in BD Cytofix™ buffer (Cat. No. 554655), permeabilized, and stained with Alexa Fluor® 488 anti-Human Nanog monoclonal antibody (pseudo colored green) at 2.5 µg/mL. Cell nuclei were counter-stained with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software. Permeabilization was done using BD™ Phosflow Perm/Wash buffer I (Cat. No. 557885) for this antibody; Triton™ X-100 is also suitable for permeabilization.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Tested During Development

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
557702	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Triton is a trademark of the Dow Chemical Company.
8. 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.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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)

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007; 318(5858):1917-1920. (Biology)