Technical Data Sheet

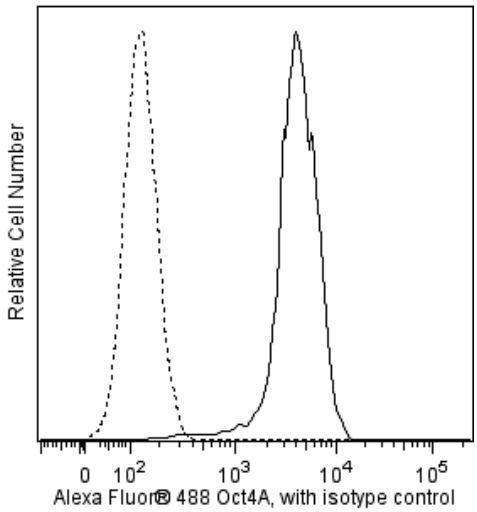
Alexa Fluor® 488 Mouse anti-Oct3/4 (Human Isoform A)

Product Information

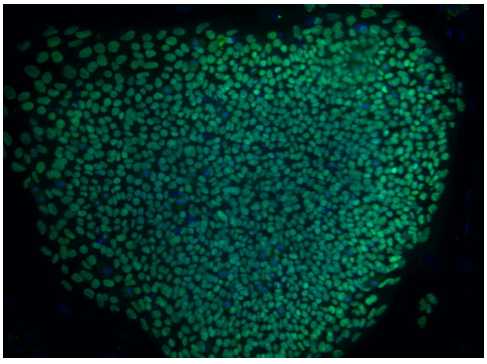
Material Number:	**561628**
Alternate Name:	Oct3/4A, Oct-3A, OTF-3, NF-A3, OTF4, POU5F1, MGC22487
Entrez Gene ID:	5460, 18999
Size:	50 tests
Vol. per Test:	5 µl
Clone:	O50-808
Immunogen:	Human Oct3/4 Isoform A Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Tested: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Development of a multicellular organism from a single fertilized egg is regulated by the coordinated activity of DNA transcription factors. Oct3/4, a member of the POU family of transcription factors, functions in pluripotent cells of early embryonic stem (ES) cell lines and embryonal carcinomas (EC). The human POU5F1 gene can encode various splice variants, two of which are Oct3/4A and Oct3/4B. Both isoforms share identical POU DNA-binding and C-terminal domains but differ in their N-terminal domain. The N-terminal domain of Oct3/4B is inhibitory to the DNA binding domain and therefore cannot stimulate transcription of Oct3/4-dependent genes. Oct3/4B can be detected in both pluripotent and some differentiated cell types in both the nucleus and cytoplasm, but its function is unclear. There is not an equivalent to Oct3/4B in mouse. Oct3/4A is expressed in the nucleus and has been demonstrated to orchestrate the transcription of Oct3/4-dependent genes. It has been demonstrated that the expression of Oct3/4 isoforms can vary greatly in different cell types, and discrimination of these is crucial for assessing Oct3/4 expression and function. The O50-808 monoclonal antibody recognizes human Oct3/4 Isoform A and mouse Oct3/4.



Analysis of Oct3/4 Isoform A on human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) were harvested, fixed in BD Cytofix™ fixation buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) and stained with matching concentrations of either Alexa Fluor® 488 Mouse IgG1, κ isotype control (Dashed line, Cat. No. 557782) or Alexa Fluor® 488 Mouse anti-Oct3/4 (Human Isoform A) monoclonal antibody (solid line). The histograms were derived from gated events based on light scattering characteristics for the H9 cell line. Flow cytometry was performed on a BD LSR™ II flow cytometry system.*



Immunofluorescent staining of Oct3/4 Isoform A in human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) passage 33 grown on irradiated mouse fibroblasts were fixed with BD Cytofix™ fixation buffer (Cat. No. 554655), permeabilized, and stained with Alexa Fluor® 488 Mouse anti-Oct3/4 (Human Isoform A) 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. The cells were permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050); 1x BD Perm/Wash™ Buffer (Cat No. 554723) is also suitable for permeabilization.*

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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
Immunofluorescence	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
557782	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	50 tests	MOPC-21
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. 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.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. *Cell.* 1990; 60(3):461-472. (Biology)

Pan G, Thomson JA. Nanog and transcriptional networks in embryonic stem cell pluripotency. *Cell Res.* 2007; 17:42-49. (Biology)

Rosfjord E, Scholtz B, Lewis R, Rizzino A. Phosphorylation and DNA binding of the octamer binding transcription factor Oct-3. *Biochem Biophys Res Commun.* 1995; 212(3):847-853. (Biology)

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