

Technical Data Sheet

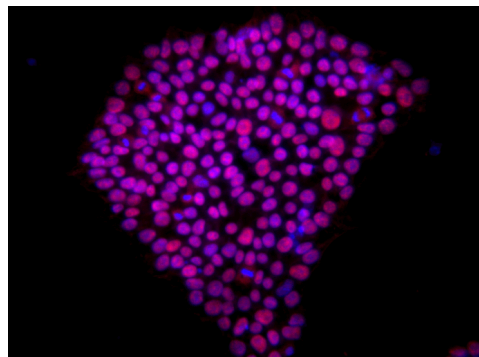
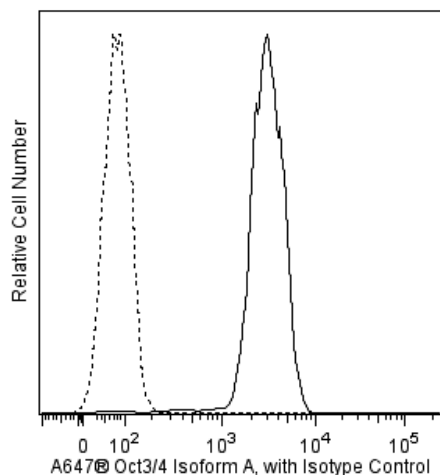
Alexa Fluor® 647 Mouse Anti-Oct3/4 (Human Isoform A)

Product Information

Material Number:	562252
Alternate Name:	Oct3/4A, Oct-3A, OTF-3, NF-A3, OTF4, POU5F1, MGC22487
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 Testing: Human Tested in Development: Mouse
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) passage 42 grown in mTESR™ 1 medium (StemCell Technologies) on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) were harvested with Accutase™ (Cat. No. 561527), fixed with BD Cytotfix™ fixation buffer (Cat. No. 554655), permeabilized with BD BD Phosflow™ Perm Buffer III (Cat. No. 558050), and stained with matching concentrations of either Alexa Fluor® 647 Mouse anti-Oct3/4 (Human Isoform A) monoclonal antibody (solid line) or Alexa Fluor® 647 mouse IgG1, κ isotype control (Clone MOPC-21, Cat. No. 557714, dashed line). The histograms were derived from gated events based on light scattering characteristics of 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 43 grown in mTESR™ 1 medium (StemCell Technologies) on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) were fixed with BD Cytotfix™ fixation buffer (Cat. No. 554655), permeabilized, and stained with Alexa Fluor® 647 Mouse anti-Oct3/4 (Human Isoform A) monoclonal antibody (pseudo-colored red) 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); 0.1% Triton is also suitable for permeabilization.

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
354277	BD Matrigel™ hESC-qualified Matrix, 5 ml vial	NA	(none)
561527	Accutase™ Cell Detachment Solution	100 ml	(none)
557714	Alexa Fluor® 647 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. 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® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry 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.
10. All other brands are trademarks of their respective owners.

References

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