Technical Data Sheet

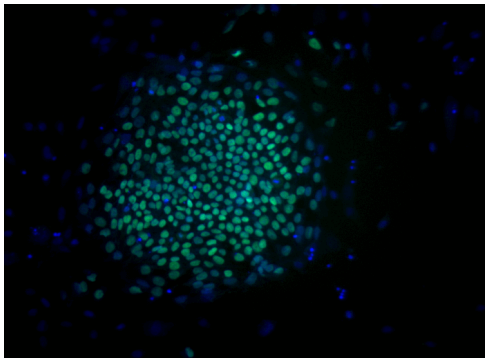
Alexa Fluor® 488 Mouse anti-Oct3/4

Product Information

Material Number:	**560217**
Alternate Name:	Oct3, OTF3, Oct4, OTF4, POU5F1
Size:	100 tests
Vol. per Test:	5 µl
Clone:	40/Oct-3
Immunogen:	Mouse Oct3 aa. 252-372 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Confirmed by western blot using purified antibody (Cat. No. 611202 or 611203) and by Bioimaging: 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 cell 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 cell (ES) lines and embryonal carcinomas (EC). Other members of the POU family include Oct1, Oct2, Pit-1, and unc-86. The POU domain, a 150-amino acid region that determines binding specificity, is conserved among these proteins and consists of 3 subdomains: POU-specific A and B subdomains and a homeobox-like subdomain. Oct3/4 is expressed in undifferentiated cells, but is lost as cells are induced to differentiate. Oct3/4 is not expressed in adult tissues. The interaction of Oct3/4 with SOX2, another embryonic transcription factor, produces an active complex that regulates expression of genes such as Nanog, UTF1, and FGF4. Although Oct3/4 is specifically phosphorylated on serine residues, this modification is not required for DNA binding, but may affect its transactivation potential. Thus, Oct3/4 is a transcription factor that plays an important role in determining early steps of embryogenesis and differentiation.



Immunofluorescent staining of human ES cell line. *H9 cells (WiCell, Madison, WI) were grown on mouse epithelial feeder cells in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219). After overnight culture, the cells were fixed, permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723) and stained with Alexa Fluor® 488 Mouse anti-Oct3/4 (pseudo-colored green) and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 Bioimager System with a 10x objective and merged using BD Attovision™ software. This antibody also stained human EC NCCIT (ATCC, Cat. No. CRL-2073), mouse EC F9 (ATCC, Cat. No. CRL-1720), and mouse ES-E14TG2a (ATCC, Cat. No. CRL-1821) cells. It also works with Triton™ X-100 and methanol permeabilization (see Recommended Assay Procedure).*

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

Bioimaging	Routinely Tested

Recommended Assay Procedure:

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 (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).

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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.
    Triton is a trademark of The Dow Chemical Company.
    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. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	488/10	515 LP	Fura/FITC
BD Pathway 435	482/35	536/40	FF506

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

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