

Technical Data Sheet

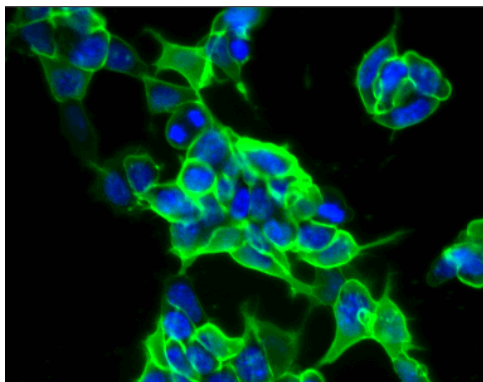
Alexa Fluor® 488 Mouse anti-SSEA-1

Product Information

Material Number:	560172
Alternate Name:	3-FAL, X-hapten, LeX antigen, CD15
Size:	100 tests
Vol. per Test:	5 µl
Clone:	MC480
Immunogen:	Mouse Teratocarcinoma Cell Line
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	Mouse, Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The MC480 monoclonal antibody reacts with Stage-Specific Embryonic Antigen-1 (SSEA-1), which is a terminal carbohydrate epitope (3-fucosyl-N-acetyllactosamine or 3-FAL) on glycoproteins and lactose series glycolipids. SSEA-1 is related to Lewis blood group antigens and is found in a variety of embryonic and adult tissues and cancers. As its name implies, the expression of SSEA-1 is stage-specific and can be used to characterize embryonic cells and monitor their differentiation. However, its expression pattern differs in the human and mouse. In the human, SSEA-1 is not found on embryonic stem (ES) cells, embryonic inner cell mass (ICM), or teratocarcinoma (embryonal carcinoma or EC) cells. As human EC and ES cells undergo differentiation, SSEA-1 expression is upregulated. In the adult, the same epitope is expressed as CD15 on granulocytes and monocytes, but not lymphocytes or dendritic cells. In the mouse, SSEA-1 is found on EC, ES, and primordial germ cells, 8-cell to blastocyst embryos, ICM, and on subpopulations of cells in the adult central nervous system, including stem cells. In contrast to the human, SSEA-1 expression is reduced as mouse EC and ES cells undergo differentiation.



Immunofluorescent staining of mouse ES cell line.
ES-E14TG2a cells (ATCC CRL-1821) were cultured, fixed, and stained with Alexa Fluor® 488 Mouse anti-SSEA-1 (pseudo-colored green) according to the Recommended Assay Procedure. Counter-staining was with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer using a 20X objective and merged using BD Attovision™ software. This antibody also stained F9 cells (ATCC CRL-1720; mouse embryonal carcinoma). Methanol and Triton X100 permeabilization agents are not recommended for use with this antibody.

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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes

Application

Bioimaging	Routinely Tested
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Recommended Assay Procedure:

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- 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.

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- 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 Cytotfix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
- Remove the fixative from the wells, and wash the wells twice with 100 µl of 1× PBS.
- Dilute the antibody 1:10 in 1× PBS, and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
- Remove the diluted antibody, and wash the wells twice with 100 µl of 1× PBS.
- 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.
- View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ cell analyzers are:

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

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554655	Fixation Buffer	100 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)

Product Notices

- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 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.
- 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.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 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.
- Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Capela A, Temple S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron*. 2002; 35:865-875. (Biology)

Childs RA, Pennington J, Uemura K, et al. High-molecular-weight glycoproteins are the major carriers of the carbohydrate differentiation antigens I, i and SSEA-1 of mouse teratocarcinoma cells. *Biochem J*. 1983; 215:491-503. (Clone-specific: Immunofluorescence, Western blot)

Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. *J Anat*. 2002; 200:249-258. (Clone-specific: Flow cytometry)

Henderson JK, Draper JS, Baillie HS, et al. Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. *Stem Cells*. 2002; 20:329-337. (Clone-specific: Flow cytometry, Immunofluorescence)

Kannagi R, Nudelman E, Lavery SB, Hakomori S. A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. *J Biol Chem*. 1982; 257(24):14865-14874. (Clone-specific)

Solter D, Knowles BB. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc Natl Acad Sci U S A*. 1978; 75(11):5565-5569. (Immunogen: Cytotoxicity, Radioimmunoassay)

Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145-1147. (Clone-specific: Immunocytochemistry (cytospins))