BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

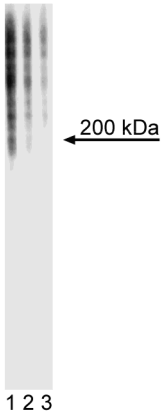
Purified Mouse anti-SSEA-1

Product Information

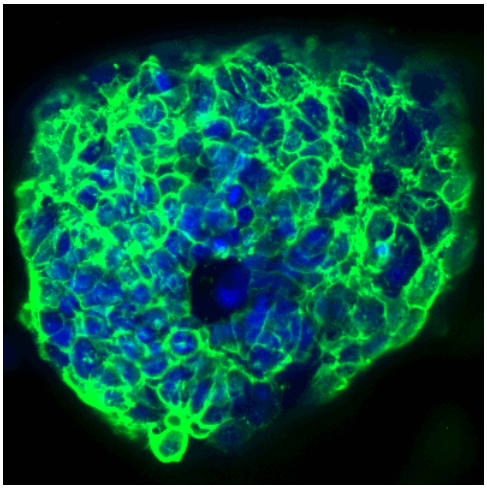
Material Number:	**560079**
Alternate Name:	3-FAL, X-hapten, LeX antigen, CD15
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	MC480
Immunogen:	Mouse Teratocarcinoma Cell Line
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	Human, Mouse
Target MW:	multiple, >200 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MC480 monoclonal antibody reacts with *S*tage-*S*pecific *E*mbryonic *A*ntigen-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.



Western Blot analysis of SSEA-1 in mouse ES cell line. *Lysate from ES-E14TG2a cells (ATCC CRL-1821) was probed with Purified Mouse anti-SSEA-1 monoclonal antibody at titrations of 1.0 (lane 1), 0.5 (lane 2), and 0.25 µg/ml (lane 3). High-molecular-weight molecules bearing the SSEA-1 epitope are identified above 200 kDa.*



Immunofluorescent staining of mouse ES cell line. *ES-E14TG2a cells were cultured, fixed, and stained with Purified Mouse anti-SSEA-1 monoclonal antibody (pseudo-colored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 647 goat anti-mouse Ig (Invitrogen) and 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.*

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Preparation and Storage

Store undiluted at 4°C.

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

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Reported
Immunocytochemistry (cytospins)	Reported
Immunofluorescence	Reported
Radioimmunoassay	Reported
Immunochemistry	Reported
Cytotoxicity	Reported

Recommended Assay Procedure:

Bioimaging

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 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 Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and wash the wells twice with 100 µl of 1× PBS.
4. Dilute the antibody in 1× PBS, and stain the cells by adding 50 µl of the diluted antibody to each well and incubating for 1 hour at RT.
5. Remove the diluted antibody, and wash the wells three times with 100 µl of 1× PBS.
6. Remove the PBS, dilute the second-step reagent in 1× PBS, and stain the cells by adding 50 µl of the diluted second-step reagent to each well and incubating for 1 hour at RT.
7. Remove the diluted second-step reagent, and wash the wells twice with 100 µl of 1× PBS.
8. 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.
9. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. 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.
4. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.

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, Levery 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))