Technical Data Sheet

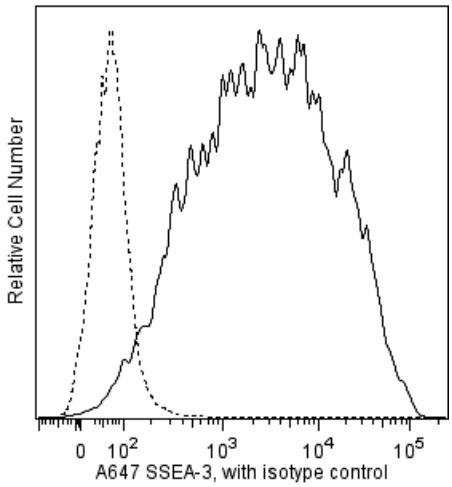
Alexa Fluor® 647 Rat Anti-SSEA-3

Product Information

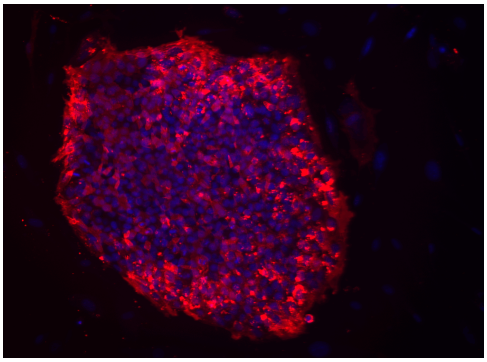
Material Number:	**561145**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	MC-631 (also known as MC631)
Immunogen:	Mouse Embryos
Isotype:	Rat (F344) IgM
Reactivity:	QC Testing: Human Reported: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The MC-631 monoclonal antibody reacts with **S**tage-**S**pecific **E**mbryonic **A**ntigen-**3** (SSEA-3), a carbohydrate epitope on the major ganglioside, but not the neutral glycolipid, of mouse embryos and human teratocarcinoma cells. As its name implies, the expression of SSEA-3 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-3 is found on teratocarcinoma (embryonal carcinoma or EC), embryonic inner cell mass (ICM), and embryonic stem (ES) cells, and erythrocytes. As human stem cells undergo differentiation, SSEA-3 expression diminishes. In the mouse, SSEA-3 is found on oocytes, ova, zygotes, early cleavage-stage embryos, early blastocysts, ICM, primitive endoderm, and adult kidney, but not on EC or ES cells.



Flow cytometric analysis of SSEA-3 expression on human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) passage 37 grown on irradiated mouse embryonic fibroblasts were harvested and stained with either Alexa Fluor® 647 rat anti-SSEA-3 antibody (solid line) or an Alexa Fluor® 647 rat IgM, κ isotype control (Clone R4-22, Cat. No.560892, dashed line). Flow cytometry was performed on a BD™ LSR II flow cytometry system.*



Immunoflourescent staining of SSEA-3 on human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) passage 33 grown on irradiated mouse embryonic fibroblasts were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and stained with Alexa Fluor® 647 rat anti-SSEA-3 monoclonal antibody (pseudo-colored red) at 10 µg/mL. Cell nuclei were stained with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ software.*

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.

BD Biosciences

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Application Notes

Application

Flow cytometry	Routinely Tested
Bioimaging	Tested During Development
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

For Bioimaging, see the protocol at http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp.

Suggested Companion Products

Catalog Number	Name	Size	Clone
560892	Alexa Fluor® 647 Rat IgM, κ Isotype Control	0.1 mg	R4-22
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 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. An isotype control should be used at the same concentration as the antibody of interest.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. 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.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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: Cytotoxicity, Flow cytometry, Immunocytochemistry (cytospins))

Kannagi R, Cochran NA, Ishigami F, et al. Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. *EMBO J.* 1983; 2(12):2355-2361. (Clone-specific: Immunofluorescence, Radioimmunoassay)

Shevinsky LH, Knowles BB, Damjanov I, Solter D. Monoclonal antibody to murine embryos defines a stage-specific embryonic antigen expressed on mouse embryos and human teratocarcinoma cells. *Cell.* 1982; 30:697-705. (Immunogen: Cytotoxicity, Immunofluorescence, Immunoprecipitation, Radioimmunoassay)

Son YS, Park JH, Kang YK, et al. Heat shock 70-kDa protein 8 isoform 1 is expressed on the surface of human embryonic stem cells and downregulated upon differentiation. *Stem Cells.* 2005; 23:1502-1513. (Clone-specific: Flow cytometry, Immunocytochemistry (cytospins))

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))