BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

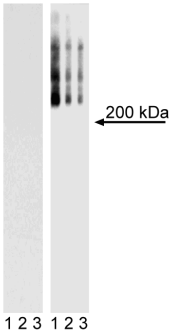
Purified Mouse anti-Human TRA-1-81 Antigen

Product Information

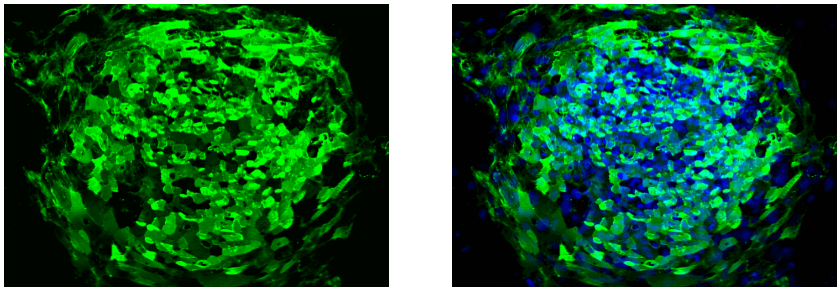
Material Number:	**560072**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	TRA-1-81
Immunogen:	Human Embryonal Carcinoma Cell Line
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	Confirmed: Human Reported: Rhesus Monkey
Target MW:	multiple, >200 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TRA-1-81 monoclonal antibody reacts with a pluripotent-stem-cell-specific epitope on a high-molecular-weight transmembrane glycoprotein. The TRA-1-81 antigen is an epitope on the same keratan sulfate core molecule, podocalyxin, as 4 other distinct antigens on tumor-derived cell lines, TRA-1-60, GCTM2, K4, and K21. The expression of TRA-1-81 antigen is stage-specific and can be used to characterize embryonic cells and monitor their differentiation. The antigen is found on teratocarcinoma (embryonal carcinoma or EC), embryonic inner cell mass (but not morula or trophoblast), and embryonic stem (ES) cells. As human EC and ES cells undergo differentiation, expression of TRA-1-81 antigen is lost.



Western Blot analysis of TRA-1-81 in mouse and human ES cell lines. *Lysates from ES-E14TG2a mouse ES cells (ATCC CRL-1821, left blot) and H9 human ES cells* (WiCell, Madison, WI, right blot) were probed with Purified Mouse anti-Human TRA-1-81 Antigen monoclonal antibody at titrations of 2.0 (lanes 1), 1.0 (lanes 2), and 0.5 µg/ml (lanes 3). High-molecular-weight molecules bearing the TRA-1-81 epitope are identified above 200 kDa in the human ES cells. Expression of the TRA-1-81 epitope has not been reported in mouse ES cells. *The H9 cells were cultured on a mitomycin C-treated mouse embryonic fibroblast feeder layer [MEF (CF-1), ATCC SCRC-1040] that maintains the undifferentiated state of the ES cells. The lysate was made from a mixture of the 2 cell types, the majority of which were H9 cells.*



Immunofluorescent staining of human ES cell line. *The H9 cell line (WiCell, Madison, WI) was cultured, fixed, and stained with Purified Mouse anti-Human TRA-1-81 Antigen monoclonal antibody (pseudo-colored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 647 goat anti-mouse Ig (Invitrogen). The left image shows the plasma membrane staining by the TRA-1-81 mAb, and the right image shows TRA-1-81 with counter-staining of the nuclei by Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer using a 10X objective and merged using BD Attovision™ software.*

Preparation and Storage

Store undiluted at 4°C.

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

BD Biosciences

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Reported
Immunocytochemistry (cytospins)	Reported
Immunoprecipitation	Reported
Radioimmunoassay	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.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(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

Andrews PW, Banting G, Damanov I, Arnaud D, Avner P. Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells. *Hybridoma.* 1984; 3(4):347-361. (Immunogen: Immunofluorescence, Immunoprecipitation, Radioimmunoassay)

Badcock G, Pigott C, Goepel J, Andrews PW. The human embryonal carcinoma marker antigen TRA-1-60 is a sialylated keratan sulfate proteoglycan. *Cancer Res.* 1999; 59:4715-4719. (Clone-specific: Immunoprecipitation, 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)

Schopperle WM, DeWolf WC. The TRA-1-60 and TRA-1-81 human pluripotent stem cell markers are expressed on podocalyxin in embryonal carcinoma. *Stem Cells.* 2007; 25:723-730. (Clone-specific: Immunofluorescence, Western blot)

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))

Thomson JA, Kalishman J, Golos TG, et al. Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci U S A.* 1995; 92:7844-7848. (Clone-specific: Immunocytochemistry (cytospins))