Technical Data Sheet

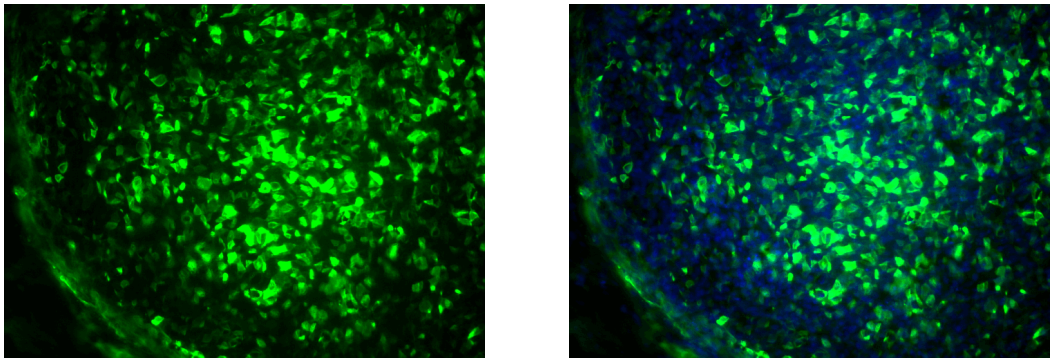
Alexa Fluor® 555 Mouse anti-Human TRA-1-60 Antigen

Product Information

Material Number:	**560121**
Alternate Name:	TRA-1-60(R)
Size:	100 tests
Vol. per Test:	5 µl
Clone:	TRA-1-60
Immunogen:	Human Embryonal Carcinoma Cell Line
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	QC Testing: Human Reported: Rhesus Monkey
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The TRA-1-60 monoclonal antibody reacts with the neuraminidase-resistant form of a pluripotent-stem-cell-specific epitope on a high-molecular-weight transmembrane glycoprotein. The TRA-1-60 antigen is a sialylated epitope on the same keratan sulfate core molecule, podocalyxin, as 4 other distinct antigens on tumor-derived cell lines, TRA-1-81, GCTM2, K4, and K21. The expression of TRA-1-60 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. TRA-1-60 antigen is released into the serum of patients bearing testicular tumors containing EC cells. As human EC and ES cells undergo differentiation, expression of TRA-1-60 antigen is lost. Expression of TRA-1-60 antigen has also been observed on a rhesus monkey ES cell line (Thomson et al, 1995).



Immunofluorescent staining of human ES cell line. *The H9 cell line (WiCell, Madison, WI) was cultured, fixed, and stained with Alexa Fluor® 555 Mouse anti-Human TRA-1-60 Antigen monoclonal antibody (pseudo-colored green) according to the Recommended Assay Procedure. The left image shows the plasma membrane staining by the TRA-1-60 mAb, and the right image shows TRA-1-60 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. If permeabilization is required for staining additional markers, we recommend the use of cold 90% methanol or BD™ Phosflow Perm Buffer III (Cat. No. 558050).*

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® 555 under optimum conditions, and unreacted Alexa Fluor® 555 was removed.

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 the wells twice with 100 µl of 1× PBS, and fix the cells by adding 100 µl of fresh 3.7%

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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 conjugate to each well and incubating for 1 hour at RT.
5. Remove the diluted antibody, and wash the wells twice with 100 µl of 1× PBS.
6. 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.
7. 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	100 tests	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. 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.
2. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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))