Technical Data Sheet

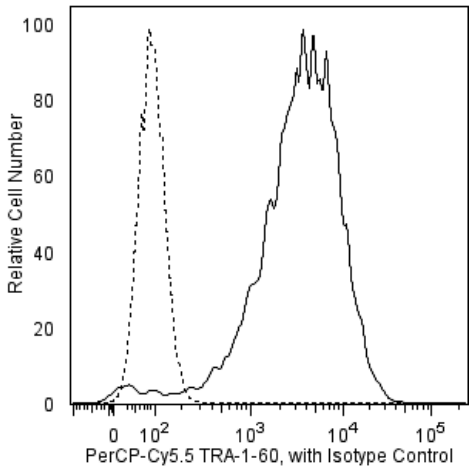
PerCP-Cy™5.5 Mouse Anti-Human TRA-1-60 Antigen

Product Information

Material Number:	**561573**
Alternate Name:	TRA-1-60(R)
Size:	50 tests
Vol. per Test:	5 µl
Clone:	TRA-1-60
Immunogen:	Human Embryonal Carcinoma Cell Line
Isotype:	Mouse (BALB/c) IgM, κ
Reactivity:	QC Tested: 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).



Flow cytometric analysis of PerCP-Cy™5.5 anti-TRA-1-60 on human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) passage 52 grown in mTESR™1 media (StemCell Technologies) on BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277) were harvested with Accutase™ (Cat. No. 561527) and stained with PerCP-Cy™5.5 Mouse anti-Human TRA-1-60 (solid line) or a PerCP-Cy™5.5 Mouse IgM, κ Isotype Control (Clone G155-228, Cat. No.560857, dashed line) Flow cytometry was performed on a BD™ LSR flow cytometry system.*

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 with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Flow cytometry	Routinely Tested

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Suggested Companion Products

Catalog Number	Name	Size	Clone
561527	Accutase™ Cell Detachment Solution	100 ml	(none)
354277	BD Matrigel™ hESC-qualified Matrix	5.0 ml	(none)
560857	PerCP-Cy™5.5 Mouse IgM, κ Isotype Control	0.1 mg	G155-228

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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
4. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
5. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
6. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
7. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
11. 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)

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)

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)

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)

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)

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

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)

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