invitrogen™ | MOLECULAR PROBES®

PRODUCT INSERT

MONOCLONAL ANTIBODY TO THE MOUSE CD154 ANTIGEN

Product	Form	Volume	Antibody*	Excitation (nm)	Peak Emission (nm)	Matching Isotype Controls	
HMCD15401	FITC	1.0 ml	500 µg	488	525	Hamster IgG FITC	HM01
HMCD15404	R-PE	1.0 ml	100 µg	488	575	Hamster IgG R-PE	HM04

PRODUCT DESCRIPTION

Hamster monoclonal antibody to the mouse CD154 antigen

Clone: MR1

Isotype: Armenian hamster IgGκ

Lot No.: See label **Expiration:** See label

Buffer: Phosphate buffered saline (PBS)

Preservatives: 0.1% ***sodium azide.*** Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

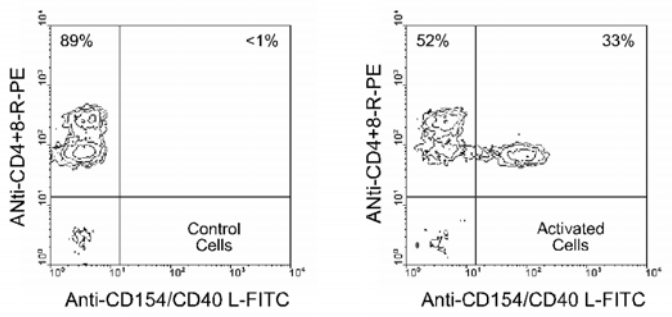
Stabilizer: Sucrose.

PRODUCT CHARACTERIZATION

Antigen Specificity: CD154, formerly known as CD40 ligand and gp39, is a type II integral membrane protein and a member of the tumor necrosis factor (TNF) family of ligands.[1-7] It is an important accessory molecule in T cell-B cell costimulatory interactions, and is expressed predominantly on activated $CD4^+$ T lymphocytes. It is also present on the surface of activated Th0, Th1, and Th2 T cell clones. Its expression is transient and cyclosporin-sensitive.[6] The MR1 monoclonal antibody binds to murine CD154 with high affinity, blocks binding to CD40, and blocks CD154 function.[1, 5] Administration of this antibody to mice blocks the ability to mount primary and secondary immune responses to TD antigens, yet does not alter the immune response to TI antigens.[4]

Research Applications:

- Flow cytometry [1, 5]
- Immunoprecipitation [1]
- *In vivo* and *in vitro* blockage of CD154 function [1, 3, 5]



Partially purified spleen T cells from BALB/c mice were incubated with either hamster IgG or plate-bound hamster anti-mouse CD3ε (clone 145-2C11) for 7 hours at 37°C. The cells were then harvested, double-stained with anti-CD154/CD40L-FITC and anti-CD4+8-R-PE, and analyzed by flow cytometry.

Note: Flow cytometric data shown may not necessarily have been generated using the enclosed lot of reagent. For this reason, and due to differences in flow cytometers and cytometer settings, results may vary from those illustrated above. It is suggested that investigators titrate reagents to determine optimal conditions for use in their systems.

STORAGE & HANDLING

Store reagents at 2-8°C. Light exposure should be avoided for fluorochrome-conjugated reagents. Use dim light during handling, incubation with cells and prior to analysis. It is recommended that cells be analyzed within 18 hours of staining. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted.

PRODUCT QUALITY CONTROL

To ensure lot-to-lot consistency, each batch of monoclonal antibody is tested by flow cytometry to conform to characteristics of a standard reference reagent. From this testing it is recommended that between 0.1 and 0.2 µg of antibody be used per 1 x 10^6 cells in a 100 µl staining volume. Because conditions may vary, it is recommended that each investigator determine the optimal amount of antibody to be used for each application.

* The amount of antibody is determined by measuring the optical density using a spectrophotometer. The antibody titer is verified by immunofluorescent staining and flow cytometric analysis.

REFERENCES:

1. Noelle, R.J., M. Roy, D.M. Shepherd, I Stamenkovic, J.A. Ledbetter, and A. Aruffo. 1992. *Proc. Natl. Acad. Sci. USA* 89:6550.
2. Aruffo, A., M. Farrington, D. Hollenbaugh, X. Li, A. Milatovich, S. Nonoyama, J. Bajorath, L.S. Grosmaire, R. Stenkamp, and M. Neubauer. 1993. *Cell* 72:291.
3. Hollenbaugh, D., L.S. Grosmaire, C.D. Kullas, N.J. Chalupny, S. Braesch-Andersen, R.J. Noelle, I. Stamenkovic, J.A. Ledbetter, and A. Aruffo. 1992. *EMBO J.* 11:4313.
4. Foy, TM., DM Shepherd, F.H. Durie, A. Aruffo, J.A. Ledbeter, and R.J. Noelle. 1993. *J. Exp. Med.* 178:1567.
5. Foy, T.M., D.M. page, T.J. Waldschmidt, A. Schoneveld, J.D. Laman, S.R. Masters, L. Tygrett, J.A. Ledbetter, A. Aruffo, and E. Claassen. 1995. J. Exp. Med. 182:1377.
6. Roy, M., T.J. Waldschmidt, A. Aruffo, J.A. Ledbetter, and R.J. Noelle. 1993. *J. Immunol.* 151:2497.
7. Armitage, R.J., W.C. Fanslow, L. Stockbine, T.A. Sato, K.N. Clifford, B.M. Macduff, D.M. Anderson, S.D. Gimpel, T. Davis-Smith, and C.R. Maliszewski. 1992. *Nature* 357:80.

FOR RESEARCH USE ONLY. . . NOT FOR THERAPEUTIC OR IN VITRO DIAGNOSTIC USE

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

PI L13029 (Rev 02/09) DCC-09-0140