

## Technical Data Sheet

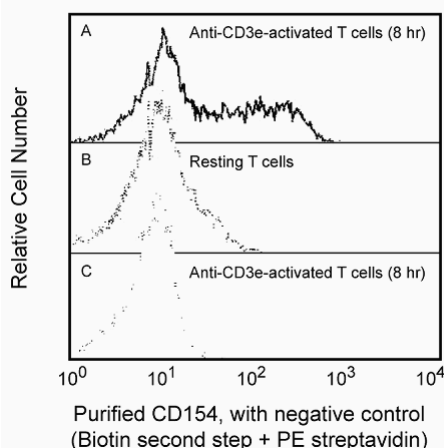
## Purified Hamster Anti-Mouse CD154

## Product Information

<b>Material Number:</b>	553656
<b>Alternate Name:</b>	CD40 Ligand, gp39
<b>Size:</b>	0.5 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	MR1
<b>Immunogen:</b>	Activated mouse Th1 clone D1.6
<b>Isotype:</b>	Armenian Hamster IgG3, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

## Description

The MR1 antibody reacts with CD154 (CD40 Ligand, gp39), an accessory molecule expressed on activated T helper (CD4+) lymphocytes. CD154 has also been detected on other types of leukocytes, including CD8+ T cells, medullary thymocytes, activated CD4+ NK-T cells, and human NK cells. CD154 plays an important role in costimulatory interactions between T and B lymphocytes and between antigen-presenting cells and lymphocytes, regulating the immune response at multiple levels. MR1 mAb inhibits *in vitro* activation of B lymphocytes by T helper cells by blocking interaction of gp39 with CD40. *In vitro* interactions of T cells and antigen-presenting cells can also be blocked by the MR1 antibody. *In vivo* treatment with MR1 antibody blocks the development of experimental autoimmune disease, inhibits formation of germinal centers and generation of memory B cells, reduces T-lymphocyte responses to allogeneic cells and allografts, prevents intrathymic deletion of self-reactive T lymphocytes, and disrupts antigen-specific T-cell responses.



**CD154 expression on activated and resting T lymphocytes.** BALB/c spleen T cells, purified on a T Cell Enrichment Column (R&D Systems), were cultured for 8 hours in the presence (Panels A and C) or absence (Panel B) of plate-bound 500A2 antibody (anti-CD3e, Cat. No. 553238). They were stained with purified MR1 antibody (Panels A and B), followed by biotinylated mouse anti-hamster IgG, Cat. No. 554010, then Streptavidin-PE, Cat. No. 554061, (Panels A, B, and C). Flow cytometry was performed on a BD FACScan™ Flow Cytometry System.

## Preparation and Storage

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

Store undiluted at 4° C.

## Application Notes

## Application

Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Reported
Blocking	Reported

## Recommended Assay Procedure:

For the detection of mouse CD154 on activated peripheral T cells, it is strongly recommended that T cells be purified before activation. Mouse CD154 is transiently expressed on the surfaces of activated normal T cells and certain T cell clones with a maximal level detected 6-8 hours

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post-activation. Activation with immobilized anti-CD3e mAb (e.g., 145-2C11, Cat. No. 557306/553058, or 500A2, Cat. No. 553238) is sufficient to induce CD154 expression on CD4+ cells. It has been reported that CD8+ cells express CD154 only in response to PMA/Ionomycin treatment. Therefore, for detection of CD154, it is crucial to utilize the proper activation stimuli and to stain cells at the optimal time for CD154 expression. We recommend the use of biotinylated mouse anti-hamster IgG cocktail (Cat. No. 554010) followed by a "bright" second-step reagent, such as Streptavidin-PE (Cat. No. 554061), for optimal detection of CD154.

## Suggested Companion Products

Catalog Number	Name	Size	Clone
553238	Purified Hamster Anti-Mouse CD3e	0.5 mg	500A2
554010	Biotin Mouse Anti-Armenian and Syrian Hamster IgG Cocktail	0.5 mg	(none)
554061	PE Streptavidin	0.5 mg	(none)
557306	Purified Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
551386	Purified Hamster IgG3, $\kappa$ Isotype Control	0.5 mg	E36-239

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [wwwbdbiosciences.com/pharminggen/protocols](http://wwwbdbiosciences.com/pharminggen/protocols) for technical protocols.
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. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at [http://wwwbdbiosciences.com/pharminggen/hamster\\_chart\\_11x17.pdf](http://wwwbdbiosciences.com/pharminggen/hamster_chart_11x17.pdf).
5. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

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