

Technical Data Sheet

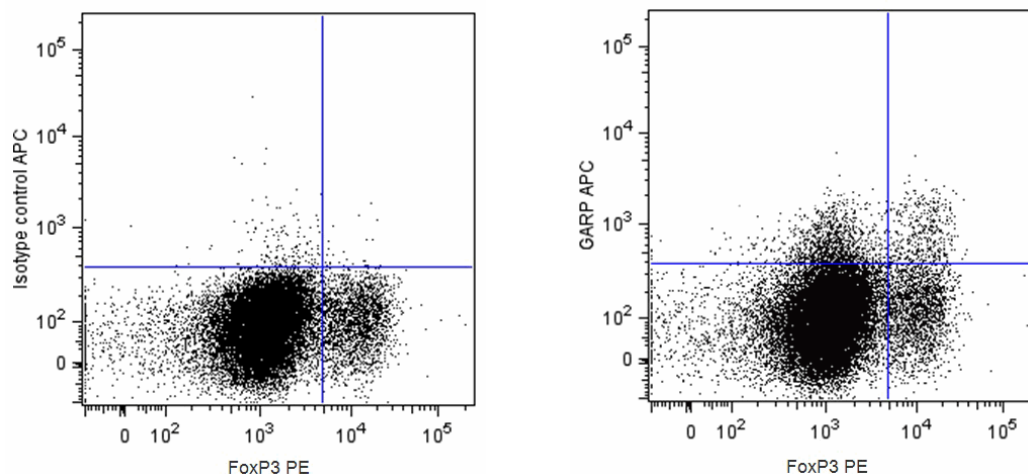
APC Mouse anti-Human GARP

Product Information

Material Number:	562341
Alternate Name:	LRRC32; Garpin; Glycoprotein A repetitions predominant
Entrez Gene ID:	2615
Size:	100 tests
Vol. per Test:	5 µl
Clone:	7B11 (also known as CMSSC-7B11)
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 7B11 (also known as CMSSC-7B11) monoclonal antibody specifically binds to human GARP (Glycoprotein A repetitions predominant). The *LRRC32* (Leucine rich repeat containing 32) gene encodes the 662 amino acid-residue, 80 kDa transmembrane GARP glycoprotein that has an extracellular region composed primarily of 20 leucine-rich repeats. GARP is specifically expressed on Treg cells activated through the T cell receptor (TCR). Ectopic expression of GARP in human naïve T cells inhibited their proliferation and cytokine secretion upon TCR activation. Remarkably, GARP over-expression in naïve T cells induced expression of FoxP3 and endowed them with a partial suppressive function. The extracellular, but not the cytoplasmic region, of GARP was necessary for these functions. GARP serves as a receptor for latent TGF-β which may play a role in the suppressive action of Treg cells. GARP is also expressed on platelets and other tissues, however the function on these cells is not known.



Multicolor flow cytometric analysis of GARP expressed on activated human peripheral blood lymphocytes. Human peripheral blood mononuclear cells (PBMC) were activated (12 hours) with plate-bound Purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and Purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725). The activated PBMC were washed and stained with either APC Mouse IgG2b κ Isotype Control (mouse IgG2b, Cat. No. 555745) or APC Mouse anti-Human GARP (Cat. No. 562341). The cells were then fixed, permeabilized and stained with BD Horizon™ V450 Mouse Anti-Human FoxP3 (Cat. No. 560459) as per the recommended protocol. Two-color flow cytometric dot plots showing the correlated expression patterns of FoxP3 versus the Ig Isotype Control (Left Panel) or GARP (Right Panel) were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer 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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

Flow cytometry

Routinely Tested

BD Biosciences

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Recommended Assay Procedure:**Suggested Staining Procedure for APC anti-Human GARP Antibody:**

1. Harvest PBMC after stimulation (12 hours) with Purified NA/LE Mouse Anti-Human CD3 (Cat. No. 555329) and Purified NA/LE Mouse Anti-Human CD28 (Cat. No. 555725).
2. Wash the cells twice with stain buffer (eg. BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656).
3. Stain 1×10^6 cells either with the APC anti-Human GARP antibody (Cat. No. 562341) or with APC Mouse IgG2b, κ Isotype Control (Cat. No. 555745) for 30 minutes on ice, protected from light.
4. Wash cells twice with stain buffer.
5. Stain for FoxP3. Refer to the Technical Data Sheet of BD Horizon™ V450 Mouse Anti-Human FoxP3, Cat. No. 560459, for detailed FoxP3 staining protocol.

In brief,

- a. Add 2 ml of 1 X FoxP3 buffer A to the cell pellet.
- b. Centrifuge and incubate in 0.5 ml of buffer C for 30 minutes.
- c. Wash twice with stain buffer and stain with V450 Anti-Human FoxP3 antibody (Cat. No. 560459) for 30-45 minutes.
- d. Wash twice with stain buffer and acquire on the Flow cytometer.

Note: BD Pharmingen™ Human FoxP3 Buffer Set (Cat. No. 560098) is a suggested companion product.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555745	APC Mouse IgG2b κ Isotype Control	100 tests	27-35
554656	Stain Buffer (FBS)	500 ml	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
560459	V450 Mouse Anti-Human FoxP3	120 tests	259D/C7
552843	Anti-Mouse Ig, κ /Negative Control (FBS) Compensation Particles Set	6.0 ml	187.1
555899	Lysing Buffer	100 ml	(none)
560098	Human FoxP3 Buffer Set	100 tests	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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.
3. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. An isotype control should be used at the same concentration as the antibody of interest.
7. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).

References

Ollendorff V, Noguchi T, deLapeyriere O, Birnbaum D. The GARP gene encodes a new member of the family of leucine-rich repeat-containing proteins. *Cell Growth Differ.* 1994; 5(2):213-219. (Biology)

Stockis J, Colau D, Coullie PG, Lucas S. Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. *Eur J Immunol.* 2009; 39(12):3315-3322. (Biology)