PERFORMANCE DATA SHEET

2635

Monoclonal anti-human GARP(LRRC32)*



mAb name/Clone: ANC8C9 *Isotype:* Mouse IgG2aκ

Immunogen: Recombinant human GARP

CATALOG#: 277-820 (Preservative-free)

QUANTITY: 100 μg CONCENTRATION: 1.0 mg/ml

INFORMATION: Human GARP (Glycoprotein A Repetitions Predominant/ Leucine Rich Repeat Containing 32) is a roughly 80 kd transmembrane cell surface molecule expressed on a variety of tissue types, including a subset of activated T regulatory cells. GARP covalently binds latent TGF-beta 1 serving as a regulatory reservoir(1). Antibody ANC8C9 binds to recombinant GARP in EIA and to cell surface GARP expressed on a subset of FOXP3 positive cells.

References: 1) R Wang, T A Springer, et al. (2012) Mol Biol Cell 23(6): 1129-1139.

STORAGE CONDITIONS: Store at $2 - 5^{\circ}C$. Open under aseptic conditions. Freeze/Thawing is not recommended.

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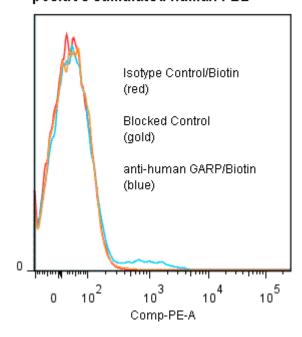
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl.

PRODUCTION: Antibody was Protein A purified from (low FBS containing) tissue culture supernatant. Purity was >95% Immunoglobulin by SDS-PAGE and contains less than 1% Bovine Immunoglobulin. Product was 0.2 µm filtered and vialed under aseptic conditions.

PERFORMANCE: (FACS analysis) Ficoll prepared human PBMC were stimulated in culture overnight with immobilized anti-human CD3 antibody (cat #144-020), anti-human CD28 mAb (cat #177-020), and human IL-2. Cells were harvested and 5 x 10⁵ cells per tube were washed and pre incubated ~5 minutes with 20 µl human IgG at 250 µg/ml (to block non specific binding) after which they were incubated 45 minutes on ice with 80 µl of anti-CD4/APC conjugate (cat #147-060) and anti-human GARP/Biotin (Cat #277-030) at a concentration of 5 ug/ml. Cells were washed twice and incubated with 50 ul of 2º reagent Streptavidin/PE (Catalog #260-050), after which they were washed three times, fixed and analyzed by FACS, gating on CD4 positive lymphoid cells. A net 4.7 % sub population of gated cells stained positive with a mean shift of **1.59** \log_{10} fluorescent units when compared to a Mouse IgG2a/Biotin isotype control (Catalog # 281-030). binding was blocked when cells were pre incubated with 50 µg/ml unlabeled anti-human GARP mAb.

Binding of anti-GARP/Biotin to CD4 positive stimulated human PBL



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