

PERFORMANCE DATA SHEET

2216

Monoclonal anti-human GARP (LRRC32)/FITC*

mAb name/Clone: ANC8C9

Isotype: Mouse IgG2ak

Immunogen: Recombinant human GARP

CATALOG#: 277-040

QUANTITY: 120 tests

VOLUME IN VIAL: 0.2ml

WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5 x 10⁵-cell test)

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°C. Freeze/Thawing is not recommended. Protect from light.

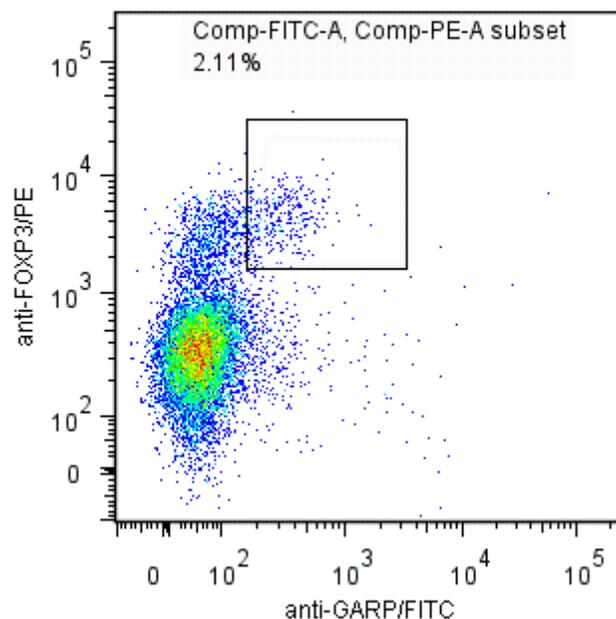
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: _____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was reacted with FITC. Unconjugated FITC was separated from antibody/FITC conjugate by desalting column. The antibody/FITC conjugate is at a concentration of **0.25 mg/ml** with a Fluorescein/IgG molar ratio of 6.6.

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/FITC at dilution factor of **1:50** (5 µg/ml). Cells were washed twice, fixed, permeabilized and co stained with anti-FOXP3/PE after which they were washed twice and analyzed by FACS, gating on CD4 positive lymphoid cells. A net **2.1%** sub population of FOXP3 positive cells stained positive with a mean shift of **0.94 log₁₀** fluorescent units when compared to a Mouse IgG2a/FITC isotype control (Catalog # 281-050). This binding was blocked when cells were pre incubated with 50 µg/ml unlabeled anti-human GARP mAb.

Binding of anti-GARP/FITC to CD4 positive PBL



**Research Use Only. Not for use in Diagnostic procedures.*