

## Monoclonal anti-human GARP(LRRC32)/Biotin\*

**mAb name/Clone:** ANC8C9

**Isotype:** Mouse IgG2ak

**Immunogen:** Recombinant human GARP

**CATALOG#:** 277-030

**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°C. Freeze/thawing not recommended.

**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% NaN<sub>3</sub> (as a preservative).

**PRODUCTION:** Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to >95% mouse immunoglobulin by SDS-PAGE (<1% bovine immunoglobulin), and reacted with NHS-Biotin. Unconjugated Biotin was removed from conjugate using a desalting column.

**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 10<sup>5</sup> 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 µg/ml. Cells were washed twice and incubated with 50 µl of 2<sup>o</sup> 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<sub>10</sub> fluorescent units when compared to a Mouse IgG2a/Biotin isotype control (Catalog # 281-030). This binding was blocked when cells were pre incubated with 50 µg/ml unlabeled anti-human GARP mAb.

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

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

