PERFORMANCE DATA SHEET
2732

Monoclonal anti-human CD272(BTLA)/R-PE*

mAb name/Clone: **ANC5A5**
Isotype: Mouse IgG1κ
Immunogen: Recombinant soluble human CD272

CATALOG#: 372-050
QUANTITY: 120 tests **VOLUME IN VIAL: 0.2 ml**
WORKING DILUTION: 1:50 (or use 1.6µl of concentrated stock per 5 x 105-cell test)

INFORMATION: Human CD272 (BTLA, B and T Lymphocyte Attenuator) is a member of the Immunoglobulin superfamily and has sequence homology to PD-1 and CTLA4. It is expressed on T and B lymphocytes and other hemopoetic lineages. Engagement of this molecule by its ligand, CD270(HVEM, a TNF superfamily member) can down regulate activated T and B cell responses. CD272 levels on antigen specific CD8+ T cells have been reported to decrease in viral specific, but not melanoma specific activated lines (1). Polymorphism of the CD272 molecule has been linked to rheumatoid arthritis (3).
Antibody from clone ANC5A5 binds to CD272 on the cell surface of human PBMC in Flow cytometry and to recombinant CD272-muIg (cat #542-020) in EIA. This antibody ***does not*** block binding of Biotinylated CD270(HVEM)-muIg(cat #531-030) to CD272-muIg in EIA, but appears to ***enhance*** this interaction. Antibodies ANC6E9(cat #272-020) and ANC5A5(cat #372-020) each bind a distinct epitope of CD272 and are suitable as a matched pair for EIA using ANC6E9 as capture and Biotinylated ANC5A5(cat# 372-030) as detector.

References: 1) Derre' L, DE Speiser, et al. (2010) *J Clin Invest* **120**(1):157-167. 2) Otsuki N, Azuma M, et al. (2006) *Biochem Biophys Res Com* **344**(4): 1121-1127. 3) Lin SC, Chan CH, et al. (2006) J Biomed Sci 13(6): 853-860. 4) Pasero C, D Olive, et al. (2009) *Curr Mol Me*d **9**(7): 911-927. 5) Gavrieli M, KM Murphy, et al. (2006) *Adv Immunol* **92**: 152-185.

STORAGE CONDITIONS: ***Store at 2 - 5^{O}C.*** **Do not freeze! Protect from light.**

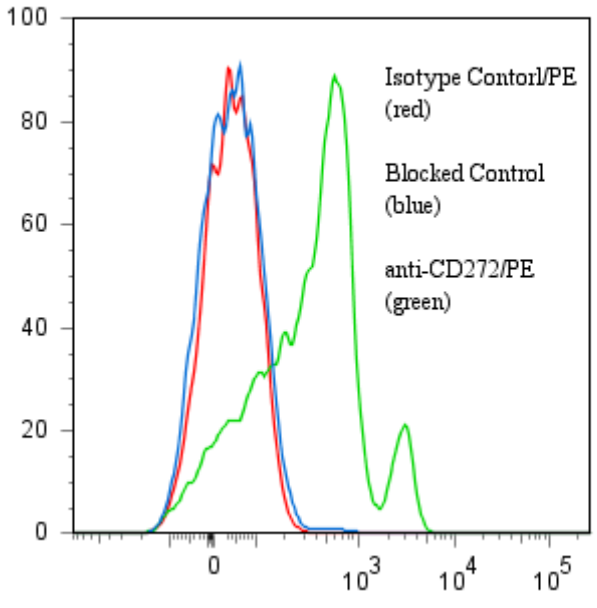
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN_3 (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at **500 µg/ml** with an A_{565}/A_{280} ratio of 2.99.

PERFORMANCE: Five x 10^5 ficoll prepared human peripheral blood mononuclear cells (PBMC) per tube were pelleted and pre incubated 5 minutes with 20µl of human IgG at 300µg/ml, after which they were washed and incubated 45 minutes on ice with 80 µl of anti-CD272/R-PE at a dilution factor of **1:50** (10 µg/ml). Cells were washed three times, fixed and analyzed by FACS using a lymphoid gate. Cells stained positive with a mean shift of **1.15** $\log_{10}$ fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog #278-050) at a similar concentration. Binding was blocked when cells were pre incubated 10 minutes with 20 µl of 0.5 mg/ml anti-CD272(clone ANC5A5) antibody (Catalog #372-020).

Binding of anti-CD272/PE (clone ANC5A5) to human PBMC (lymphoid gate)



****This Product is intended for Laboratory Research use only.*** R-Phycoerythrin (R-PE) is covered under patents: U.S. 4,520,110; European 76,695 and Canadian 1,179,942.

Ancell Corporation P.O. Box 87 Bayport, MN 55003-0087 USA
Phone: Toll free 800-374-9523 or 651-439-0835 Fax: 651-439-1940