PERFORMANCE DATA SHEET

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Monoclonal anti-human CD272(BTLA) *

mAb name/Clone: ANC6E9 *Isotype:* Mouse IgG1κ

Immunogen: Recombinant soluble human CD272

CATALOG#: 272-020 QUANTITY: 100 μg

CONCENTRATION: 1.0 mg/ml

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 ANC6E9 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 *blocks* binding of Biotinylated CD270(HVEM)-muIg(cat #531-030) to CD272-muIg in EIA. Antibodies ANC6E9 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) <u>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 Med* **9**(7): 911-927. 5) Gavrieli M, KM Murphy, et al. (2006) *Adv Immunol* **92**: 152-185.</u>

STORAGE CONDITIONS: *Store at 2 - 5^oC*. Freeze/Thawing is 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, 0.5 mg/ml Gentamicin Sulfate (as a preservative).

Binding of anti-CD272 mAb (clone ANC6E9)

PRODUCTION: Antibody was Protein A purified from (low FBS containing) tissue culture supernatant. Purity was >95% Immunoglobulin by SDS-PAGE with less than 1% Bovine Immunoglobulin.

PERFORMANCE: Five x 10⁵ 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 antibody at **10 μg/ml**. Cells were washed twice and incubated with 2⁰ reagent Goat anti-Mouse IgG/FITC (Catalog #232-011) after which they were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **1.17** log₁₀ fluorescent units when compared to a Mouse IgG1 negative control (Catalog #278-010) at a similar concentration. Binding was blocked when reagent was pre incubated with a 10-fold excess of recombinant CD272-muIg (cat #542-020).

HGAM/FITC to human PBMC

Isotype Control (red)

Blocked Control (blue)

anti-CD272 mAb (green)

10⁰ 10¹ 10² 10³

*This Product is intended for Laboratory Research use only.

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