

# PERFORMANCE DATA SHEET

2440

## Monoclonal anti-human CD279(PD-1)/Biotin\*

**mAb name/Clone:** ANC4H6

**Isotype:** Murine IgG1κ

**Immunogen:** Recombinant human CD279

**CATALOG#:** 279-030

**QUANTITY:** 100 µg

**CONCENTRATION:** 1.0 mg/ml

**INFORMATION:** Human CD279 (PD-1, Programmed death 1) is a 55 kd Ig superfamily member with similarity to CD28 and CD152(CTLA-4). It is expressed on activated T and B and myeloid cells, and engagement by its ligands PD-L1 (CD274, B7-H1) or PD-L2 (B7-DC) can inhibit proliferation and cytokine expression. In mice blockade of PD-1 ligand interaction has been used to augment T cell anti cancer responses (3,4,5).

Anti-CD279 antibody clone ANC4H6 binds to CD279 present on PHA activated PBMC in FACS, and binds to recombinant CD279 in EIA. Pre incubation of recombinant CD279-coated plates with ANC4H6 blocks binding of biotinylated recombinant CD273 in EIA.

**References:** 1) Ishida et al. (1992) *EMBO J* **11**(11):3887-95. 2) Carreno BM, M Collins, et al. (2001) *Annu Rev Immunol* **20**:29. 3) MA Curran, Allison JP, et al. (2010) *PNAS* **107**(9): 4275-80. 4) Mangsbo SM, TH Totterman, et al. (2010) *J Immunotherapy* **33**(3):225. 5) Hirano F, L Chen, et al. (2005) *Canc Res* **65**: 1089.

**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% Immunoglobulin by SDS-PAGE, and reacted with NHS-Biotin.

Unconjugated Biotin was removed from conjugate using a desalting column.

**PERFORMANCE:** Ficoll prepared human peripheral blood mononuclear cells (PBMC) were stimulated 5 days in culture with 5 µg/ml Phytohemagglutinin (PHA). Five x 10<sup>5</sup> cells per tube were pre incubated 10 minutes with 250 µg/ml human IgG (to block nonspecific binding) after which they were incubated 45 minutes on ice with 80 µl of product at a concentration of **10 µg/ml**. Cells were then washed twice and incubated 45 minutes with 20 µl of 2<sup>o</sup> reagent Streptavidin/R-PE (Catalog #253-050), after which they were washed three times, fixed and analyzed by FACS. Cells stained positive with a mean shift of **1.04 log<sub>10</sub>** fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030) at a similar concentration. Binding was blocked when reagent was pre incubated 10 minutes with a ten-fold excess of recombinant CD279-muIg.

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

**Binding of anti-CD279(PD-1)/Biotin +SA/PE to stimulated human PBMC**

