

PERFORMANCE DATA SHEET***Monoclonal anti-human CD133(AC133)/R-PE*******mAb name/Clone:*** ANC9C5***Isotype:*** Mouse IgG1k***Immunogen:*** WERI-Rb-1 human retinoblastoma cells**CATALOG#:** 363-050**QUANTITY:** 120 tests**OLUME IN VIAL:** 0.2 ml**WORKING DILUTION:** 1:50 (80ul/test)

INFORMATION: Human CD133 is a 117kd 5 transmembrane protein expressed by a subset of hematopoietic stem cells found in blood and some other tissues. Increased expression of CD133 may be a predictor of decreased prognosis in patients with metastatic cancer(3).

Antibody ANC9C5 recognizes epitope 1 of human CD133(AC133) present on full length CD133 transfectants, Y-79 retinoblastoma, and other cell types.

References: 1) Miraglia S, Buck DW, et al. (1997) Blood 90(12): 5013-5021. 2) Shmelkov SV, Rafii S, et al. (2005) Int J Biochem Cell Biol 37(4): 715-9. 3) Mehra N, Voest EE, et al. (2006) Clin Canc Res 12 (16): 4859-66.

STORAGE CONDITIONS: *Store at 2 - 5°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: _____

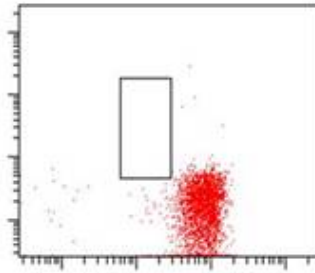
BUFFER: 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN₃ (as a preservative).

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 **200 mg/ml** with an A₅₆₅/A₂₈₀ ratio of 3.1.

PERFORMANCE: Antibody ANC9C5 was tested for its ability to bind to full length CD133 transfectants Y-79 retinoblastoma cells, and other CD133+ cells in flow cytometry.

Five x 10⁵ ficoll prepared human peripheral blood leukocytes per tube were pre incubated with 20 ml of 250 ug/ml human Ig (to block non specific binding) after which they were incubated 45 minutes on ice with 80 ul of anti-CD133/PE at a dilution factor of **1:50** (4ug/ml) and costained with anti-CD45/FITC (cat #196-040). Cells were washed three times, fixed and analyzed by FACS. A propidium iodide control tube was used to help accurately set the live cell gate. A net **0.1%** sub population of the cells which costained CD45(lo)+ was positive for CD133 with a mean shift of 1.3 log₁₀ fluorescent units when compared to a Mouse IgG1/R-PE negative control (Catalog # 278-050) at a similar concentration. PE positive binding for this subpopulation was blocked when cells were pre incubated 10 minutes with 20 ul of 0.5 mg/ml of unconjugated anti-CD133 antibody (Catalog #363-020).

Mouse IgG1/PE Isotype Control



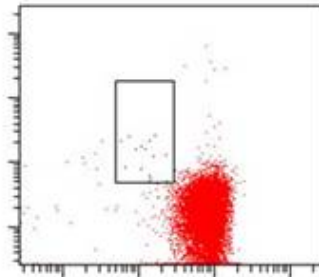
anti-CD133/PE binds to a subpopulation of Human PBL

Fresh ficoll prepared human PBL

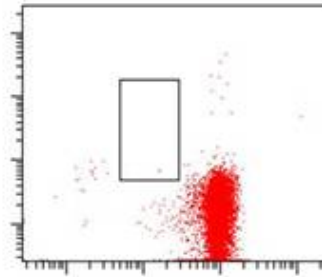
Live gate was set on propidium iodide non reactive lymphoid population

0.1% CD133+ subpopulation

anti-CD133/PE



Cells preblocked with excess of anti-CD133 Ab



anti-CD45/FITC

****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.