

MONOCLONAL ANTIBODY

# PE labeled CD44

Code No.	Clone	Subclass	Quantity
D140-5	15C6	Mouse IgG2a	1 mL (50 tests)

**BACKGROUND:** CD44 (H-CAM/Pgp-1/Hermes antigen/ECMR-III/HUTCH-I) is a highly glycosylated transmembrane protein expressed by lymphocytes, fibroblasts, smooth muscle cells, and epithelial cells. CD44 functions as lymphocyte adhesion molecule, acting as a matrix receptor that mediates cell adhesion to the extracellular matrix. CD44 is also involved in T-lymphocyte activation, lymphocyte homing, cell migration, and hemopoiesis. Expression of CD44 on the cell surface changes profoundly during tumor metastasis, and the transition from non-metastatic to metastatic tumor cell variants is associated with expression of CD44 variants (CD44v's), making CD44 a potential cancer marker

**SOURCE:** This antibody was purified from hybridoma (clone 15C6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c mouse splenocyte immunized with MML-1 human leukemia cells.

**FORMULATION:** 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN<sub>3</sub>.

\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with CD44 antigen on Flow cytometry.

**APPLICATION:**

Flow cytometry: 20 µL (ready for use)

\*Please refer to the data sheet (MBL code no. D140-3) for other applications.

Detailed procedure is provided in the following **PROTOCOLS.**

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cells	lymphocyte, monocyte, granulocyte	Not tested	Not tested
Reactivity on FCM	+		

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

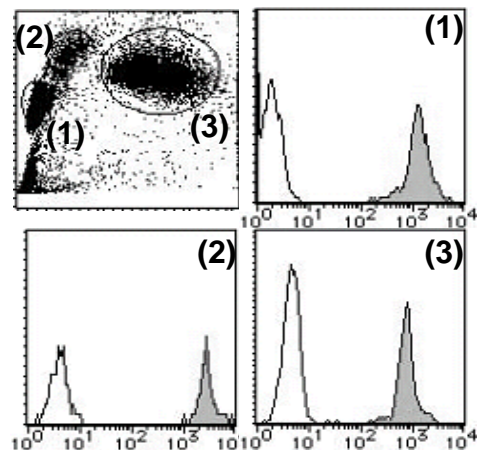
**REFERENCES:**

- 1) Kozaki, K., *et al.*, *Cancer Res.* **60**, 2535-2540 (2000)
- 2) Sugiyama, K., *et al.*, *Immunol Invest.* **28**, 185-200 (1999)

Clone 15C6 is used in these references.

**RELATED PRODUCTS:**

- D140-3 CD44 (15C6)
- D140-4 FITC labeled CD44 (15C6)



**Flow cytometric analysis of CD44 expression on lymphocyte (1), monocyte (2) and granulocyte (3).** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D140-5 to the cells.

**PROTOCOLS:**

**Flow cytometric analysis for floating cells**

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (5x10<sup>6</sup> cells/mL).
- 3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.

- 4) Add 10  $\mu\text{L}$  of normal goat serum containing 1 mg/mL normal human IgG and 0.1%  $\text{NaN}_3$  to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 20  $\mu\text{L}$  of PE labeled CD44 (15C6). Mix well and incubate for 20 minutes at room temperature.
- 6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Resuspend the cells with 500  $\mu\text{L}$  of the washing buffer and analyze by a flow cytometer.

#### **Flow cytometric analysis for whole blood cells**

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

- 1) Add 20  $\mu\text{L}$  of PE labeled CD44 monoclonal antibody (15C6) into each tube.
- 2) Add 100  $\mu\text{L}$  of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 °C).
- 3) Add 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1%  $\text{NaN}_3$ ] followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
- 5) Add 1 mL of  $\text{H}_2\text{O}$  to each tube and incubate for 10 minutes at room temperature.
- 6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 8) Resuspend the cells with 500  $\mu\text{L}$  of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; lymphocyte, monocyte, granulocyte)