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

MONOCLONAL ANTIBODY

FITC labeled CD44

Code No.	Clone	Subclass	Quantity	Concentration
D140-4	15C6	Mouse IgG2a	1 mL	50 μg/mL

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 μg IgG in 1 mL volume of PBS containing 1% BSA and 0.09% NaN_3.
*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.

APPLICATIONS:
Flow cytometry; 10 μg/mL (final concentration)
*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
Cell	KG-1	Not tested	Not tested
Reactivity on FCM	+		

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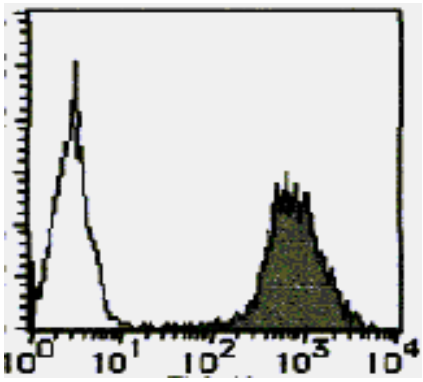
REFERENCES:
1) Kozaki, K., *et al*., *Cancer Res*. **60**, 2535-2540 (2000)
2) Sugiyama, K., *et al*., *Immunol Invest*. **28**, 185-200 (1999)

Clone 15C6 is used in these references.

RELATED PRODUCTS:
D140-3 CD44 (15C6)
D140-5 PE labeled CD44 (15C6)



Flow cytometric analysis of CD44 expression on KG-1 cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of K0140-4 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_3].
2) Resuspend the cells with washing buffer ($5x10^6$ 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 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.

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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 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; KG-1)

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 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer into each tube.
2) Add 100 μL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 ^{o}C).
3) Add 1 mL of washing buffer 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 H_2O 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 μL of the washing buffer and analyze by a flow cytometer.