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

MONOCLONAL ANTIBODY

CD44

Code No.	Clone	Subclass	Quantity	Concentration
D140-3	15C6	Mouse IgG2a κ	100 µg	1 mg/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. CD44 has a predicted molecular weight of 85-90 kDa, but glycosylation can increase the apparent to ~200 kDa.

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: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

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

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 2 µg/200 µL of cell extract from $5x10^6$ cells.
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 1-10 µg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	KG-1	Not Tested	Not Tested
Reactivity on FCM	+		

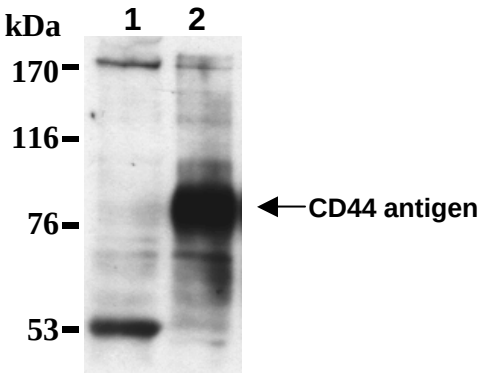
REFERENCES:

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:

D140-4 FITC labeled CD44 (15C6)
D140-5 PE labeled CD44 (15C6)



Immunoprecipitation of CD44 antigen from biotinylated KG-1 cells with mouse IgG2a (1) or D140-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and blotted biotinylated CD44 antigen on PVDF membrane was detected with HRP conjugated streptavidin.*

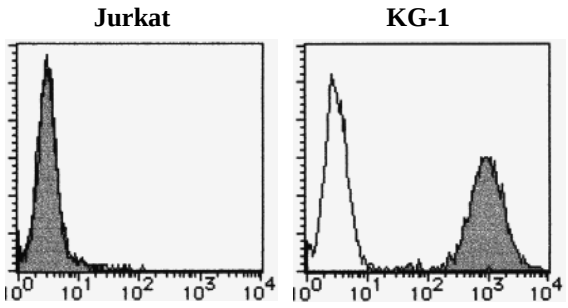
PROTOCOLS:

Immunoprecipitation

1) Wash the biotin labeled cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).

2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
8) Incubate the membrane with the 1:10,000 HRP-conjugated streptavidin (MBL; code no. IM-0309) diluted with 1% skimmed milk (in PBS, pH 7.2) for 5-30 minutes at room temperature.
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; KG-1)



Flow cytometric analysis of CD44 antigen expression on Jurkat cells and KG-1 cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D140-3 to the cells.*

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.
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) Add 30 µL of 1:40 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) 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.
9) 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 Falcon tubes or equivalents as reaction tubes for all step described below.

1) Add 50 µL of primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 µL 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 followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) 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.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature.

Remove supernatant by careful aspiration.

9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.