AM26680FC-N

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Monoclonal Antibody to CD165

Alternate names: AD2, gp37

Catalog No.: AM26680FC-N

Quantity: 50 µg

Concentration: 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.

Host / Isotype: Mouse / IgG1

Clone: AD2

Immunogen: HSB cells

Format:
State: Liquid Ig fraction
Purification: Protein-A Sepharose
Buffer System: PBS
Preservatives: 0.09% NaN3
Stabilizers: 1% BSA

Applications: Flow cytometry: 20 L (ready for use); see protocol below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with CD44.

Species Reactivity: **Tested:** Human

Add. Information: This product was originally produced by MBL International.

Storage: Store at 2 - 8 °C.
Shelf life: one year from despatch.

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

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% NaN3].
2) Resuspend the cells with washing buffer (5x106 cells/mL).

3) Add 50 L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 L of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 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 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 L of PE labeled CD44 monoclonal antibody (15C6) into each tube.
2) Add 100 L of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
3) Add 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3] 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 H2O 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 L of the washing buffer and analyze by a flow cytometer.