AM26680AF-N

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Monoclonal Antibody to CD165 - Azide Free

Alternate names: AD2, gp37

Catalog No.: AM26680AF-N

Quantity: 0.1 mg

Background: CD165 or gp37 is a cell surface molecule present on a subset of peripheral lymphocytes and monocytes and is important for adhesion of thymocytes to thymic epithelial cells.

Host / Isotype: Mouse / IgG1

Clone: AD2

Immunogen: HSB cells

Format:
State: Liquid Ig fraction
Purification: Protein-A Sepharose
Buffer System: PBS containing 50% glycerol, without preservatives

Applications: Flow cytometry: 10-20 mg/ml (final concentration).
For deteils see protocols below.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts with AD2 antigen.

Species Reactivity: **Tested:** Human

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

Storage: Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Bruggers, C.S., et al. J. Immunol. 154, 2012-2022 (1995).

Protocols: **Flow cytometric analysis for floating cells**

Protocol 1
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) Add 10 mL of normal goat serum to the cell pellet after tapping. Mix well, and incubate for 5 minutes at room temperature (20~25 oC).
3) Add 30 µL of the CD165 monoclonal antibody (10-20 mg/mL) diluted with the washing buffer. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
4) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
5) Add 30 µL of secondary antibody (1:40 FITC conjugated anti-mouse IgG) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature (20~25oC).

6) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
7) Resespend the cells with 500 mL of the washing buffer and analyze by a flow cytometer.

Protocol 2
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 PBS containing 25% normal goat serum and 0.1% NaN3 (5x10e6 cells/ml).
3) Add 20 mL of the CD165 monoclonal antibody (50 mg/mL) diluted with the washing buffer into each tube.
4) Add 50 mL of the cell suspension into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 oC).
5) Add 1 mL of the washing buffer followed by centrifugation at 500xg rpm for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
6) Resuspend the cells with 50 mL of the washing buffer.
7) Add 20 µL of secondary antibody (1:10 FITC conjugated anti-mouse IgG) diluted with the washing buffer into each tube. Mix well and incubate for 15 minutes at room temperature (20~25oC).
8) Add 1 mL of the washing buffer followed by centrifugation at 500xg for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 mL of the washing buffer and analyze by a flow cytometer.