Monoclonal Anti-human CD26/DPPIV-Fluorescein

Catalog Number: FAB1180F
Lot Number: KII03

Reagents Provided

This kit provides enough reagents for a total of 100 reactions.

Clone #: 222113

Isotype: rat IgG_{2A}

Carboxyfluorescein-conjugated rat monoclonal anti-human CD26: contains 1.0 mL of fluorescein-labeled antibody at a concentration of 50 μg/mL.

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

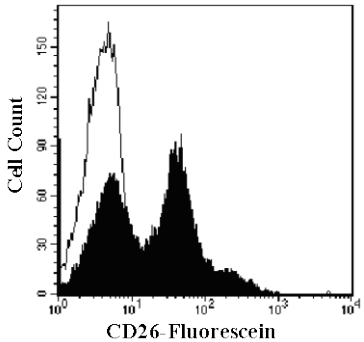
Designed to quantitatively determine the percentage of cells bearing CD26 within a population and qualitatively determine the density of CD26 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing CD26. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing the CD26 structure are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD26. Cell surface expression of CD26 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated rat anti-human CD26: Use as is; no preparation necessary.



Peripheral blood lymphocytes stained with anti-human CD26-Fluorescein (Catalog # FAB1180F, filled histogram) or isotype control (Catalog # IC006F, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. Transfer 50 μL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should be Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-CD26 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CD26 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Background Information

CD26/DPPIV (Dipeptidyl Peptidase IV) is a type II membrane protein whose extracellular domain contains a serine exopeptidase that releases Xaa-Pro dipeptides from the N-terminus of oligo-and polypeptides (1, 3). In its membrane form, CD26 is present as a noncovalently linked homodimer. A soluble form of CD26 cleaved from the cell surface is also found in serum and other body fluids (4). CD26 is constitutively expressed on epithelial cells of the intestine, prostate and kidney (5). There is a low level of expression of CD26 on resting T cells, but CD26 is upregulated on T cells, B cells and NK cells upon cellular activation (6). A higher level of CD26 expression has been found on Th1 and Th0 cells as compared to Th2 cells, suggesting a correlation with T-helper cell activity (7, 8). Once known as THAM (thymocyte activating molecule), CD26 is costimulatory for T cells (3, 9). In addition to its protease activity, CD26 also binds to collagen with low affinity suggesting a role for CD26 in lymphocyte adhesion (10). Of clinical importance, the CD26 molecule can serve as a cofactor for HIV entry in $CD4^+$ T cells (11, 12). In addition, levels of the soluble form of CD26 have been correlated with cancer progression (13), liver disease (14), kidney disease (15) and depression (16). The proteolytic activities of CD26 for neuropeptides and chemokines have been well described (17, 18).

References

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Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.