NEUROMICS

ACE/CD143-For FC (PE Labeled) Data Sheet

Catalog Number:	FC15000	**Species Reactivity:**	Human
Product Type	Mouse Monoclonal Protein G purified IgG_1 Antibody. Clone #: 171417	**Format:**	1.0 mL of PE-labeled antibody, at a concentration of 50 µg/mL.
Size:	100 Tests		
Intended Use:	Designed to quantitatively determine the percentage of cells bearing CD143 within a population and qualitatively determine the density of CD143 on cell surfaces by flow cytometry.	**Note:**	This reagent 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.
Storage:	2 -8° C		
Immunogen Sequence:	Clone #: 171417		

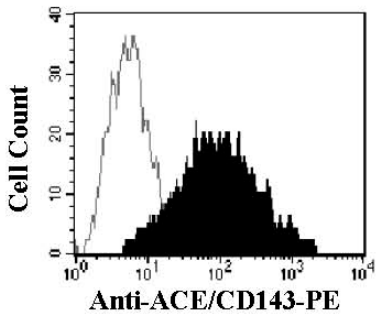
Application Notes and Protocol

Additional Reagents Required
- PBS (Dulbecco's PBS)
- BSA

Principle of the Test
Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing CD143. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing CD143 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CD143. Cell surface expression of CD143 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation
Use as is; no preparation is necessary.

Figure: Human dendritic cells, generated by culturing monocytes in the presence of IL-4 (20 ng/mL) and GM-CSF (50 ng/mL) for 5 days, were stained with anti-human ACE/ CD143-PE (filled histogram) or isotype control (open histogram).



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Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. 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. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood cells will require RBC lysis 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 x 10^6 cells/mL and 25 μL of cells (1 x 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 for 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 a rocker platform will prevent reattachment to the substrate.

Sample Staining
1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-ACE/CD134 reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-ACE/CD134 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent).*
6) 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 PE-labeled mouse IgG_1 antibody. This procedure may need to be modified, depending upon final utilization.

References

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