Monoclonal
Anti-human IL-20 Rα-Allophycocyanin

Catalog Number: FAB11762A
Lot Number: LUM02
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated monoclonal anti-human IL-20 Rα: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 173714

Ig class: mouse IgG_1

Additional Reagents Required

- 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

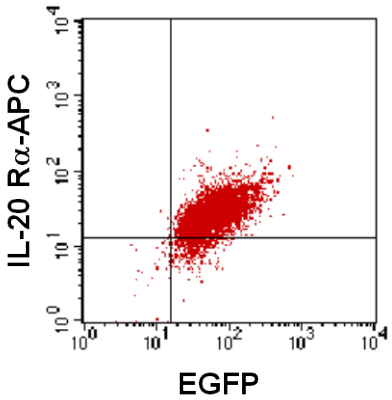
To quantitatively determine the percentage of cells expressing the cell surface receptor IL-20 Rα and qualitatively determine the density of this receptor on cell surfaces within a population by flow cytometry.

Principle of the Test

Cells are incubated with the APC-labeled monoclonal antibody, which binds cells expressing the IL-20 receptor alpha chain. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing IL-20 Rα are fluorescently stained, with the intensity of staining directly proportional to the density of IL-20 Rα. Cell surface expression of IL-20 Rα is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

APC-conjugated mouse anti-human IL-20 Rα: Use as is; no preparation necessary.



Mouse Baf3 pre-B cells transfected with human IL-20 Rα and enhanced green fluorescent protein (EGFP) stained with APC-conjugated anti-human IL-20 Rα (Catalog # FAB11762A).

Sample Preparation

Tissues: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. 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. Whole blood cells 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) are 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 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 (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated anti-human IL-20 Rα reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove any unreacted anti-IL-20 Rα 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, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
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 APC-labeled mouse IgG_1 antibody.

This procedure may need to be modified, depending upon final utilization.

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Background Information

The biologic effects of IL-20 are mediated by its binding to two heterodimeric receptors comprised of three different class II cytokine receptor chains; the IL-20 Rα plus IL-20 Rβ chains, or the IL-22 Rα plus IL-20 Rβ chains (1 - 3). IL-20 Rα, also named IL-20 R1, CRF2-8, and ZCYTOR7, is widely expressed and is detected in multiple tissues including; skin, testis, heart, placenta, salivary gland, and prostate gland (3). The expression of IL-20 Rα, together with that of IL-20 Rβ, is upregulated in psoriatic skin lesions on keratinocytes, immune cells, and endothelial cells. (3, 4) The IL-20 Rα/IL-20 Rβ receptor also functions as a mediator of IL-19, and IL-24 (2, 5). The IL-20 Rα heterodimer with the IL-10 Rβ chain also functions as a receptor complex for IL-26 (6, 7). Investigations show that the diversity in biologic effects elicited by these cytokines, although redundant in their use of receptor complexes, is likely due to the signaling mechanisms elicited by the receptor (1, 2).

References

1. Kotenko, S.V. and J.A. Langer (2004) Int. Immunopharm. **4**:593.
2. Parrish-Novak, J. *et al*. (2002) J. Biol. Chem. **277**:47517.
3. Blumberg, H. *et al.* (2001) Cell **104**:9.
4. Conti, P. *et al.* (2003) Immunol. Lett. **88**:171.
5. Dumoutier, L. *et al*. (2001) J. Immunol. **167**:3545.
6. Donnelly, R.P. *et al.* (2004) J. Leuk. Biol. **76**:314.
7. Sheikh, F. *et al*. (2004) J. Immunol. **172**:2006.

Technical Notes

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.

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