Monoclonal Anti-human IFN-γ R1-Fluorescein (CDw119)

Catalog Number: FAB673F
Lot Number: LAS02

Reagents Provided

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

Clone #: 92101

Isotype: mouse IgG1

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human IFN-γ R1: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

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 the cell surface receptor IFN-γ R1, or CDw119 or IFN-γ Rα, within a population and determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to cells expressing IFN-γ R1. Cells expressing IFN-γ R1 are fluorescently stained, with the intensity of staining directly proportional to the density of IFN-γ R1. Cell surface expression of IFN-γ R1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human IFN-γ R1: Use as is; no preparation necessary.

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. 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 a rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells should 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-human IFN-γ R1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove any unreacted anti-IFN-γ R1 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 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 murine IgG_1 antibody.

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

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Background Information

Interferon-gamma (IFN-γ) is a 17 kDa polypeptide that exerts its biological activity by interacting, in a homodimer form, with cells that bear IFN-γ receptors. The IFN-γ receptor is composed of two protein chains referred to as IFN-γ R1 (IFN-γ Rα, CDw119) and IFN-γ R2 (IFN-γ Rβ, Accessory Factor-1). The mature form of IFN-γ R1 is 472 amino acids and has a relative molecular weight of 90 kDa. IFN-γ R1 directly binds IFN-γ while a second chain is required for full biological activity (1, 2). The IFN-γ R1 does not have intrinsic kinase or phospatase activity, however, the intracellullar portion of the protein contains sequences of amino acids that allow for signaling molecules like Jak1 and Stat1 to bind to the protein (3, 4). The intracellular portion of IFN-γ R2 interacts with Jak2 (3). IFN-γ initiated cellular signaling is thought to occur following the interaction of two IFN-γ molecules with two IFN-γ R1 and two IFN-γ R2 chains (5). IFN-γ R1 binds INF-γ with an affinity of 10^{-9} to 10^{-10} M^{-1} (6). The IFN-γ R1 chain is expressed on most cells at a density of 200 - 25,000 sites/cell (5, 6). Variability in cell responses to IFN-γ may be a reflection of differential expression of IFN-γ R2 chains (7). Th1 cells, which secrete IFN-γ, lack cell surface expression of the IFN-γ R2 chain while Th2 cells, which do not produce IFN-γ, express IFN-γ R2 on their cell surface (8).

References

1. Jung, V. *et al*. (1987) Proc. Natl. Acad. Sci. USA **84**:4151.
2. Soh, J. *et al*. (1994) Cell **76**:793.
3. Bach, E.A. *et al*. (1996) Mol. Cell Biol. **16**:3214.
4. Farrar, M.A. *et al*. (1992) Proc. Natl. Acad. Sci. USA **89**:11706.
5. Bach, E.A. *et al*. (1997) Annual Rev. Immunol. **15**:563.
6. Farrar, M.A and Schrieber R.D. (1993) Ann. Rev. Immunol. **11**:571.
7. Pernis, A. *et al*. (1995) Science **269**:245.
8. Bach, E.A. *et al*. Science **270**:1215.

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.