

Reagents Provided

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

Clone #: DX2

Isotype: mouse IgG₁

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human Fas: Supplied as 50 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 Fas receptor (CD95) within a population and qualitatively 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, which binds to the cells expressing Fas. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing Fas are fluorescently stained, with the intensity of staining directly proportional to the density of Fas. Cell surface expression of Fas is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human Fas (CD95): 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. 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 x 10⁶ cells/mL and 25 µL of cells (1 x 10⁵) 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/10⁵ cells for 15 minutes at 18° - 24° C. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of fluorescein-conjugated anti-Fas reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted anti-Fas 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 IgG₁ antibody.

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

Background Information

Apoptosis was originally described as a mechanism of controlled or physiological cell death (1). It is associated with the regulation of cellular homeostasis in organs and the elimination of damaged cells or cells with deleterious reactivities from the host. Apoptosis is very common in tissues with intense hematopoietic activity (*e.g.* bone marrow and thymus) and in organs with high proliferative activity. Additionally, apoptosis has been implicated in the progression of a number of pathological conditions, including AIDS, cancer and autoimmune diseases (2, 3). Apoptosis is characterized by a variety of cellular changes including loss of membrane phospholipid asymmetry (4), chromatin condensation, mitochondrial swelling and DNA cleavage (5). The end result of these changes is a form of cell death that avoids the normal inflammatory response associated with necrosis.

Fas (CD95) is a 325 amino acid, type I transmembrane protein that structurally belongs to the TNF and NGF family of proteins (6). When Fas interacts with Fas-Ligand on cell surfaces it induces apoptosis in cells expressing Fas. Alternatively, Fas expressing cells can be induced to undergo apoptosis following crosslinking with antibodies directed against Fas (7, 8, 9). Fas is expressed on a variety of normal and tumor cells.

Anti-Fas clone DX2 was produced from mice immunized with L-cells transfected with human Fas. Clone DX2 is ideally suited for cell surface detection of Fas without inducing apoptosis due to its IgG class type and its inability to easily crosslink the Fas moiety.

References

1. Kerr, J.F.R. *et al.* (1972) *Br. J. Cancer.* **26**:239.
2. Ameisen, J.C. *et al.* (1995) *Trends Cell Biol.* **5**:27.
3. Thompson, C.B. (1995) *Science* **267**:1456.
4. Vermes, I. *et al.* (1995) *J. Immunol. Meth.* **184**:39.
5. Darzynkiewicz, Z. *et al.* (1992) *Cytometry* **13**:795.
6. Itoh, N. *et al.* (1991) *Cell* **66**:233.
7. Trauth, B.C. *et al.* (1989) *Science* **245**:301.
8. Yonehara, S. *et al.* (1989) *J. Exp. Med.* **169**:1747.
9. Cifone, M.G. *et al.* (1994) *J. Exp. Med.* **180**:1547.

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.