

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-human

Mesothelin: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 420411

Isotype: rat IgG_{2A}

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

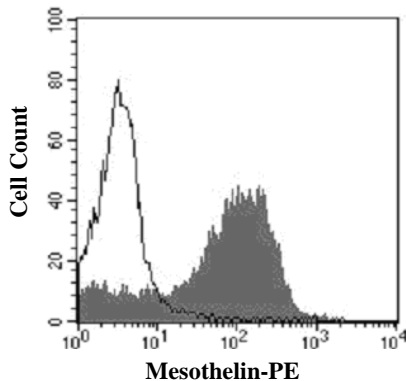
Designed to quantitatively determine the percentage of cells bearing Mesothelin within a population and qualitatively determine the density of Mesothelin on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Mesothelin. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Mesothelin are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Mesothelin. Cell surface expression of Mesothelin is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated rat anti-human Mesothelin: Use as is; no preparation necessary.



HeLa cells were stained with PE-conjugated anti-human Mesothelin (Catalog # FAB32652P, filled histogram) or isotype control (Catalog #

IC006P - open histogram).
試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775
 e-mail: reagent@funakoshi.co.jp

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred 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) 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) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates 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/10⁵ 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 x 10⁵ cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of PE-conjugated Mesothelin reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted Mesothelin 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 analysis by flow cytometry.
- 7) As a control for analysis, cells in a separate tube should be treated with PE-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

Background Information

Mesothelin is a 40 kDa glycosylphosphatidylinositol-anchored glycoprotein that is expressed on mesothelial cells in the pleura, pericardium, and peritoneum, and overexpressed in mesotheliomas, and ovarian or pancreatic adenocarcinoma. Mesothelin is a product of the CAK-1 gene, which also encodes megakaryocyte-potentiating factor (MPF). Mature human Mesothelin shares 60% amino acid identity with either mouse or rat Mesothelin. Two variant forms exist; variant one has an eight amino acid (1 kDa) insertion and is rarely expressed, while variant two is a truncated form secreted in the majority of ovarian cancers but rarely found in normal individuals.

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.