Anti-Neu5Gc Antibody Kit Protocol - Flow Cytometry

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Abstract

Anti-Neu5Gc may be used for staining cells prior to analysis by Flow Cytometry. This kit contains all the essential components needed to identify Neu5Gc on the surface of cells by flow cytometry.

Use of the blocking agent (Neu5Gc Assay Blocking Solution) provided in the kit is essential, as commonly used blocking agents invariably contain serum, or serum components, that can either inhibit detection or introduce Neu5Gc contamination.

Tissue culture-grown CHO-K1 can be used as a positive control, and human peripheral blood mononuclear cells serve as a negative control. Adherent tissue culture-grown cells should be released from the culture flasks by using 5-10mM EDTA for 10 minutes at room temperature. Other non-enzymatic methods, such as Accutase, may be used. Immediately wash cells in blocking buffer that contains a lower concentration of EDTA, and resuspend cells in blocking buffer to determine cell numbers and viability.

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Guidelines

It is assumed that the user is familiar with the general principles and practices of Flow Cytometry.

Items required but not supplied:

- Reagent grade ultra-filtered water
- Disposable cytometry tubes
- Positive Control
- Fluorescently-conjugated Secondary Antibody
- Buffer
- PBS

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Protocol

Step 1.

Prepare 0.5% Neu5Gc Assay Blocking Solution in PBS (diluent buffer).

Step 2.

The following tubes should be set up for flow cytometry analysis:

* Three (3) tubes containing cells to be examined. If choosing to use different dilutions of primary antibody, as noted in step 3, more tubes may be needed.

* Three (3) tubes containing positive control cells.

* Three (3) tubes containing negative control cells

Step 3.

Each set of tubes to be analyzed should receive an antibody treatment as follows:

* Tube 1 will contain cells that will receive no antibody, to be used as unstained control (optional but highly recommended)

* Tube 2 will contain cells that receive Control Antibody

* Tube 3 will contain cells that receive Primary Antibody

- Note: if this is the first time running this experiment, optimize by using different dilutions of primary antibody. More tubes containing sample will be needed.

NOTES
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The dilution of the antibody can range from1:200 to 1:1,000. Investigator must determine optimum dilution for cells being analyzed.

Step 4.

Wash cells by adding 1 ml cold PBS, then gently centrifuge at 4°C. Carefully remove supernatant and discard.

Step 5.

Gently resuspend cells in 100 µl of the appropriate diluted antibody as outlined above.

Step 6.

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Incubate cells on ice for at least 1 hour

DURATION
01:00:00

Step 7.

Wash cells as above

Step 8.

Gently resuspend cells in 100 µl Secondary Antibody in diluent buffer, and incubate for 1 hour on ice.

DURATION
01:00:00

Step 9.

Wash cells as before.

Step 10.

Resuspend cell pellet in 400 µl of diluent buffer

Step 11.

Run cells through the flow cytometer.

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