



# Anti-Neu5Gc Antibody Kit Protocol - Flow Cytometry V.3

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Sam Li<sup>1</sup>

<sup>1</sup>BioLegend

BioLegend

Tech. support email: [tech@biolegend.com](mailto:tech@biolegend.com)



Sam Li

BioLegend



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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 cells 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.

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

## Materials

### MATERIALS

 Phosphate Buffered Saline (PBS) **BioLegend Catalog #926201**

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



- 1 1. Prepare 0.5% Neu5Gc Assay Blocking Solution in PBS (diluent buffer).
- 2 1. 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.
- 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:** The dilution of the antibody can range from 1:200 to 1:1,000. Investigator must determine optimum dilution for cells being analyzed.

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

- 4 Wash cells by adding 1ml cold PBS, then gently centrifuge at 4°C. Carefully remove supernatant and discard.
- 5 Gently resuspend cells in 100µl of the appropriate diluted antibody as outlined above.
- 6 Incubate cells on ice for at least 1 hour.
- 7 Wash cells as above.
- 8 Gently resuspend cells in 100µl Secondary Antibody in diluent buffer, and incubate for 1 hour on ice.

**Note:** The dilution of the antibody can range from 1:200 to 1:1,000. Investigator must determine optimum dilution for cells being analyzed.
- 9 Wash cells as before.
- 10 Resuspend cell pellet in 400µl of diluent buffer.



11 Run cells through the flow cytometer.