

Flow Cytometry Analysis of HER2 Expression

INTRODUCTION

Fluorescence staining is a powerful analytical tool used to determine the expression and distribution pattern of a particular protein in cells and tissues. With flow cytometry, analysis of several protein markers can be performed simultaneously using different types of non overlapping fluorescent dyes, for example to perform population dynamics studies.

The Anti-HER2 Affibody[®] molecule is a highly specific affinity ligand that advantageously can be used for flow cytometry as a robust alternative to antibodies. The Anti-HER2 Affibody[®] molecule is available as a biotin conjugated reagent for staining with streptavidin fluorescent dye or as a fluorescein conjugated reagent, which functions as a convenient one step reagent. The Anti-HER2 Affibody[®] molecule is also available as an unconjugated reagent that is easily coupled to any thiol-activated fluorescent dye.

RESULTS

FLOW CYTOMETRY ANALYSIS OF HER2 EXPRESSION

The Oregon Green[®]-conjugated Anti-HER2 Affibody[®] molecule was used as a one step detection reagent for analysis of HER2 expression using flow cytometry. Cells from the HER2 positive human ovarian cancer cell line SK-OV-3 and the HER2-negative human neuroblastoma cell line SH-SY5Y were stained with the Oregon Green[®]-conjugated Anti-HER2 Affibody[®] molecule. As shown in figure 1a, staining with Oregon Green[®]-conjugated Anti-HER2 Affibody[®] molecule resulted in increased fluorescence intensity and the whole cell population of SK-OV-3 cells was shifted to the right (red line) compared to the control (black line). On the contrary, Anti-HER2 Affibody[®] molecule staining of the HER2 negative cell line SH-SY5Y did not cause a shift in fluorescence intensity, as shown in figure 1b.

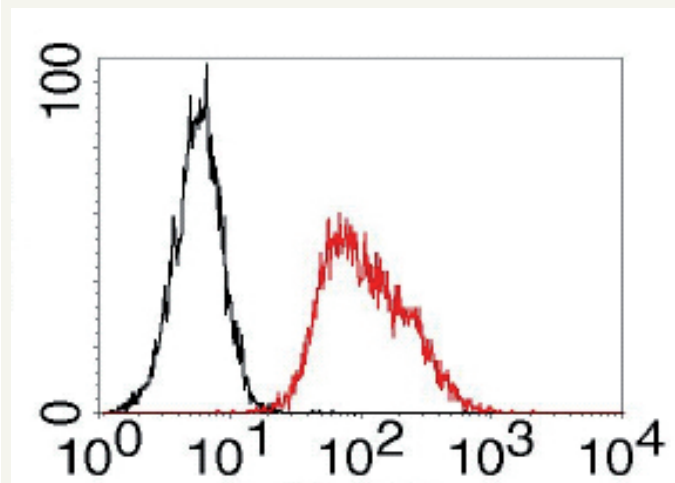


Fig. 1a. Fluorescence histogram from analysis of HER2 expressing SK-OV-3 cells. Staining with Anti-HER2 Affibody[®] molecule caused an increase in fluorescence intensity as shown by a shift to the right (red line) compared to control (black line).

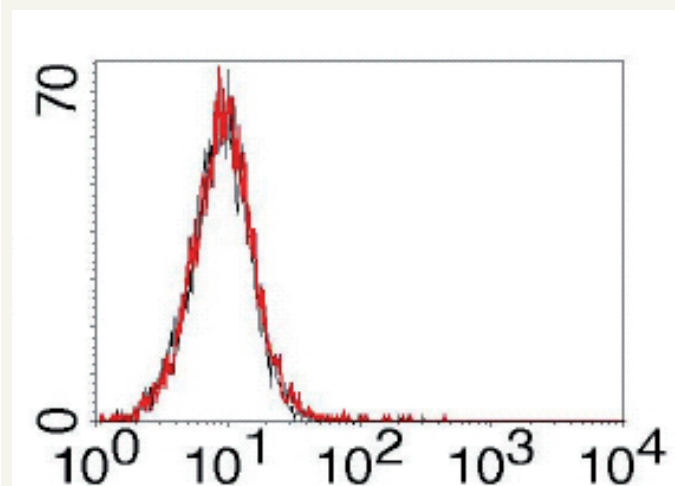


Fig. 1b. Fluorescence histogram from analysis of HER2 negative SH-SY5Y cells. Staining with Anti-HER2 Affibody[®] molecule did not cause shift in fluorescence intensity (red line) compared to control (black line).

Fluorescence Staining of Cells for Flow Cytometry Analysis

MATERIALS AND BUFFERS REQUIRED

Staining reagent:

Unconjugated Anti-HER2 Affibody[®] molecule (Affibody cat no 10.0817.01.0005)

Unconjugated Anti-HER2 Affibody[®] molecule (Affibody cat no 10.0817.01.0005)

Fluorescein conjugated Anti-HER2 Affibody[®] molecule (cat no 10.0817.03.0001)

Round-bottom tubes: 5 ml (Falcon cat no 352063)

PBS: 2.68 mM KCl, 1.47 mM KH₂PO₄, 137 mM NaCl, 8.1 mM Na₂HPO₄, pH 7.4

Fluorescent conjugation: Streptavidin ALEXA (Molecular Probes cat no S11223)

STAINING FOR FLOW CYTOMETRY

1. Prepare cells (adherent or in suspension) in PBS in an appropriate manner to obtain single cell suspensions.
Note: The need for blocking should be tested by the user. We recommend 1% BSA or 1% FCS in PBS.
2. Count cells and adjust concentration to 1 x 10⁶ cells/ml.
3. Aliquot 100 µl of cell suspension into the required number of round-bottom tubes.
4. Add an appropriate volume of conjugated Affibody[®] molecule to obtain the suitable final concentration.
Note: A final concentration of 0.1-5 µg/ml conjugated Affibody[®] molecule is recommended. The user is required to determine the optimal concentration.
5. Mix well and incubate on ice, or at +4°C, for 30 minutes.
6. Wash with 3 ml of PBS, centrifuge at 400*g for 5 minutes. Repeat once.
7. Resuspend the cells in 100 µl PBS.
8. Acquire data by using a flow cytometer.

Conjugation of Affibody[®] Molecule

INTRODUCTION

The Anti-HER2 Affibody[®] molecule is delivered with a unique C-terminal cysteine that is easily conjugated with the fluorescent dye of choice. Bright and strong staining has been obtained with the Oregon Green[®] dye, one of many dyes that are available as a thiol reactive derivative. The Affibody[®] molecule is conjugated as described by the manufacturer. However, the Affibody[®] molecules are partially dimerized due to S-S bridges formed by the C-terminal cysteine and reduction of the Affibody[®] molecule immediately prior to conjugation is therefore an absolute necessity. A brief protocol for reduction of the C-terminal cysteine is found below.

MATERIALS AND BUFFERS REQUIRED

NAP5-column: (GE Healthcare cat no 17-0853-01)

DTT: Dithiothreitol

Thiol reactive fluorescent dye: Oregon Green[®] 488 maleimide (Molecular Probes cat no O6034)

Desalting columns: (Pierce cat no 89849)

PBS: 2.68 mM KCl, 1.47 mM KH₂PO₄, 137 mM NaCl, 8.1 mM Na₂HPO₄, pH 7.4

REDUCTION OF AFFIBODY MOLECULE

1. Dissolve the lyophilized Affibody[®] molecule in PBS to obtain a final concentration of 1 mg/ml.
2. Add DTT to a final concentration of 20 mM at >pH7.5.
3. Incubate at room temperature for 2 hours.
4. Remove excess DTT by passage through a NAP5-column. The Affibody[®] molecule will re-dimerize quickly and dialysis is therefore not recommended.
5. Immediately after step 4 above, add the conjugate at the recommended molar excess and follow the protocol from the fluorescent dye manufacturer.
6. After completed conjugation and dialysis, we strongly recommend an extra desalting step using protein desalting spin columns to remove all remaining free fluorescent dye.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Affibody AB's instructions. Affibody AB's sole liability is limited to replacement of the product or refund of the purchase price. Affibody[®] products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody[®] products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Affibody AB.

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