

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

RESULTS

FLOW CYTOMETRY ANALYSIS OF EGFR EXPRESSION

The fluorescein conjugated Anti-EGFR Affibody[®] molecule was used as a one step detection reagent for analysis of EGFR expression using flow cytometry. Human squamos carcinoma A431 cells were stained with the fluorescein conjugated Anti-EGFR Affibody[®] molecule. As shown in Figure 1, staining with fluorescein conjugated Anti-EGFR Affibody[®] molecule resulted in a shift in fluorescence intensity of the cell population (red line) compared to control (black line). A two-fold titration of the fluorescein conjugated Anti-EGFR Affibody[®] molecule was performed and Figure 2 shows the mean fluorescence intensity (MFI) plotted against Affibody[®] molecule concentration resulting in a sigmoidal binding curve.

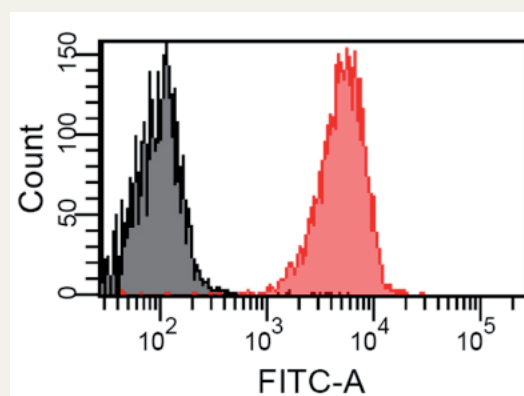


Fig. 1. Fluorescence histogram from flow cytometry analysis on EGFR expressing A431 cells. Staining with fluorescein conjugated Anti-EGFR Affibody[®] molecule caused an increase in fluorescence intensity as shown by a shift to the right (red line) compared to control (black line).

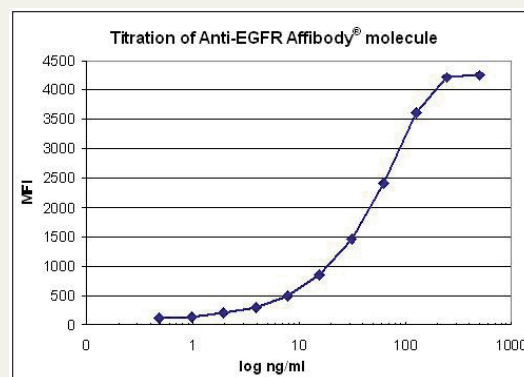


Fig. 2. Titration diagram from cytometry analysis of EGFR expressing A431 cells. The fluorescein conjugated Anti-EGFR Affibody[®] molecule was titrated on EGFR expressing A431 cells. The concentration was plotted against the mean fluorescence intensity (MFI).

Fluorescence Staining of Cells for Flow Cytometry Analysis

MATERIALS AND BUFFERS REQUIRED

Staining reagent:

Fluorescein conjugated Anti-EGFR Affibody[®] molecule (Affibody cat no 10.1886.03.0001)

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

BSA: Calbiochem (cat no 12657)

STAINING FOR FLOW CYTOMETRY

1. Prepare cells (adherent or in suspension) in PBS in an appropriate manner to obtain single cell suspensions.
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 a 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 as well as the need for blocking. However, we recommend that the fluorescein conjugated Anti-EGFR Affibody[®] molecule is diluted in 0.5% BSA in PBS.
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.
7. Repeat once.
8. Resuspend the cells in 100 µl PBS. Acquire data by using a flow cytometer.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to confirm 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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