

## Fluorescence Staining of Cell Surface EGF-Receptors

### 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 a fluorescence microscope, it is possible to follow changes in localization of a protein for example after external stimuli. With flow cytometry, analysis of several protein markers can be performed simultaneously using different types of non overlapping fluorescent dyes, performing population dynamics studies for example.

The Anti-EGFR Affibody<sup>®</sup> molecule is a specific affinity ligand that can advantageously be used for fluorescence staining of the growth receptor/tumour marker EGFR on cells. The Anti-EGFR Affibody<sup>®</sup> molecule is available as a fluorescein conjugate which functions as a convenient one step reagent. The Anti-EGFR Affibody<sup>®</sup> molecule is also available as an unconjugated reagent that is easily coupled to any thiol-activated fluorescent dye.

### RESULTS

The human squamous carcinoma A431 expresses high levels of EGFR, and this cell line was used for fluorescence staining. The A431 cells were stained with fluorescein conjugated Anti-EGFR Affibody<sup>®</sup> molecules at a concentration of 2  $\mu\text{g}/\text{ml}$ . The nuclei were counter stained with DAPI. Figure 1 shows that the fluorescein conjugated Anti-EGFR Affibody<sup>®</sup> molecule bound to A431, resulting in a bright membrane staining.

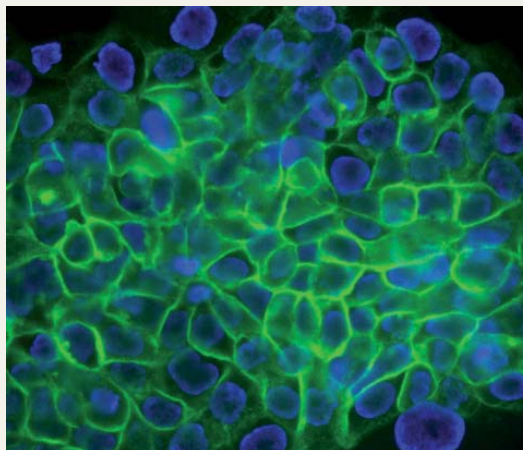


Fig. 1. A431 cells were stained with fluorescein conjugated Anti-EGFR Affibody<sup>®</sup> molecules. The staining was localized to the membrane of the EGFR-expressing A431 cells.

## Fluorescence Staining of Cell Surface EGF-Receptors

### MATERIALS AND BUFFERS REQUIRED

**Staining reagent:** Fluorescein conjugated Anti-EGFR Affibody<sup>®</sup> molecule (cat no 10.1886.03.0001)

**Multi-well slides:** (Histolab Products cat no 06278) or equivalent

**PBS:** 2.68 mM KCl, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4

**BSA:** #12657, Calbiochem

**DAPI:** (Molecular Probes cat no D1306)

**Anti-fading reagent:** (Vector Laboratories cat no H-1000)

4. Incubate in a moist chamber for 45-60 minutes at room temperature.
5. Wash the slides gently 2 times with PBS.
6. For contrast, counter stain the cell nuclei with DAPI, one droplet per well for 5 seconds and wash away DAPI excess with PBS.
7. Allow slides to dry completely, protected from light.
8. Mount with anti-fading reagent before examination in a UV microscope.

### PREPARATION OF CELLS FOR STAINING ON MULTI-WELL SLIDES

1. Adherent cells: Grow cells in a suitable medium over night on multi-well slides to obtain the flattened morphology of adherent cells. A confluence of less than 70% facilitates microscopy examination.
2. Next day, gently remove medium and fix the cells with 3% formaldehyde in PBS for at least 10 minutes in room temperature (RT).
3. Wash the slides gently 2 times with PBS.

### STAINING ON MULTI-WELL SLIDES

1. If necessary, block the slides with 1% Bovine Serum Albumin (BSA) or 1% Fetal Calf Serum in PBS for 15 minutes by adding one droplet (25-50 µl) blocking solution per well. **Note:** The need for blocking should be tested by the user. In general, Affibody<sup>®</sup> molecules show very little background staining. However, different cell lines and type of multi-well slides may behave differently and the need for blocking should therefore be tested by the user.
2. Remove the blocking solution gently. Wipe away any remaining buffer between the wells on the slide.
3. Add 25 µl (for 8 multi-well slides) of the Affibody<sup>®</sup> molecule, diluted in PBS + 0.5% BSA. A concentration in the range of 1-5 µg/ml conjugated Affibody<sup>®</sup> molecule is recommended. However, the optimal concentration should be determined by the user.

### LIMITATIONS

Warranty: Affibody<sup>®</sup> 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<sup>®</sup> products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody<sup>®</sup> 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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