

Immunofluorescence assays for epidermal growth factor receptor (EGFR) and transferrin receptor (TfR)

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INTRODUCTION

This assays allows a qualitative analysis of EGFR and TfR internalization, unveiling defects in internalization and/or subsequent trafficking steps along the endocytic pathway. Colocalization of the fluorescently-labeled ligands with markers of the different endocytic compartments allows to follow the trafficking of both receptors along the endocytic route. TfR, once internalized in the endosomal/recycling compartment (rab11-positive), is recycled back to the plasma membrane. EGFR, once it reaches the endosomes, is either recycled back to the surface or targeted for degradation into the lysosome. In HeLa cells, after 15 min it localizes with endosomal markers (e.g. EAA1); at 30-60 min it reaches the lysosomes (Lamp-1/2) and the signal starts to disappear because of degradation. Treatment with lysosomal inhibitors (such as chloroquine) causes the accumulation of intracellular EGF/EGFR complex.

MATERIALS

Binding buffer

- DMEM
- 0.1% BSA
- 20 mM Hepes

METHODS

1. Cells are plated on glass coverslips and are either transfected, infected or subjected to various treatments.
2. Cells are then serum-starved for 3/4 hours in binding buffer.
3. After starvation, cells are incubated in the presence of 0.5 µg/ml of rhomamine-EGF or 50 µg/ml of rhodamine-Tf (both from Molecular Probes) for different time points (2-120 min).
4. Wash in ice-cold PBS 1x and fix in 4% paraformaldehyde
5. Fixed cells are processed for fluorescence microscopy.

Internalisation assay with anti-EGFR 13A9 antibody (from Genentec)

1. The 13A9 antibody recognises the extracellular portion of EGFR without interfering with binding to EGF, thus allowing to follow the internalisation of the receptor independently from its ligand.
2. Cells are plated on glass coverslips and serum-starved for 3/4 hours in binding buffer.
3. Cells are incubated 1h at 4°C with 40 µg/ml of 13A9 antibodies diluted in binding buffer, in presence or absence of EGF (100 ng/ml), followed by shift at 37°C for different time points (2-120 min) in binding buffer (without the antibody).
4. After fixation, cells are permeabilized and stained with a Cy3-conjugated secondary antibody.

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