

## <sup>125</sup>I-EGF and <sup>125</sup>I-Tf internalization assays

Simona Polo and Pier Paolo di Fiore

IFOM – FIRC Institute of Molecular Oncology, Milan, Italy

### INTRODUCTION

This assay allows to measure the rate of internalization of EGFR and to calculate the endocytic rate constant ( $K_e$ , see also Wiley and Cunningham, 1982). The data obtained should be plotted as internalized/bound radioactivity (Y-axis) versus time (X-axis). The  $K_e$  ( $\text{min}^{-1}$ ) is the slope of the trendline (calculated based on linear regression). When measured at low receptor occupancy (non saturant EGF concentration) and early time-points (2-8 min),  $K_e$  represents an intrinsic property of EGFR in a given cellular system.

### MATERIALS

#### Binding buffer

- DMEM
- 0.1% BSA
- 20 mM Hepes

#### Acid wash solution pH 2.5

- acetic acid 0.2M
- NaCl 0.5M

### METHODS

1. Cells are plated into 24 well in order to have 90% of confluence the day after (in the case of HeLa, 100.000 cells). Cells are plated in triplicates for each time point, plus one well for the unspecific binding.
2. The day after, cells are serum-starved for at least 4 hours in binding buffer, and then incubated at 37°C in the presence of 1.5 ng/ml <sup>125</sup>I-EGF (PerkinElmer) or 1 µg of <sup>125</sup>I-Tf (PerkinElmer) in 300 µl of binding buffer.
3. After different time points (usually 2, 4, 6 and 8 min for initial rate), cells are put on ice, washed twice in cold PBS, and then incubated for 5 minutes at 4°C in 300 µl of acid wash solution pH 2.5. Remove the solution from the cells and measure the radioactivity. This sample represents the amount of <sup>125</sup>I-EGF or <sup>125</sup>I-Tf bound to the receptor on the cell surface.
4. Left the cells dry at room temperature for 5 minutes and then lyse with 300 µl of 1N NaOH.
5. Collect the lysate and measure the radioactivity. This sample represents the amount of internalised <sup>125</sup>I-EGF or <sup>125</sup>I-Tf

The unspecific binding is measured at each time point in the presence of an excess of non-radioactive EGF (300X) or Tf (500X). After being corrected for non-specific binding, the rate of internalisation is expressed as the ratio between internalised and surface-bound radioactivity.

To measure internalisation at high dose of EGF, the same assay is performed at one time point (4-6 min in HeLa cells) in the presence of 1.5 ng/ml of <sup>125</sup>I-EGF plus 18.5 ng/ml of cold EGF in order to reach the final concentration of 20 ng/ml. In this case, the unspecific binding is measured at each time points in the presence of 6 µg/ml of non-radioactive EGF (300X).

## BIBLIOGRAPHY

Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I. (2003) Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat Cell Biol.* 5:461-6.

Tosoni D, Puri C, Confalonieri S, Salcini AE, De Camilli P, Tacchetti C, Di Fiore PP. (2005) TTP specifically regulates the internalization of the transferrin receptor. *Cell.* 123:875-88.

Sigismund S, Woelk T, Puri C, Maspero E, Tacchetti C, Transidico P, Di Fiore PP, Polo S. (2005) Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A.* 102:2760-5.

Sigismund S., Argenzio E., Tosoni D., Cavallaro E., Polo S. and Di Fiore P.P. (2008) Clathrin-mediated internalisation is essential for sustained EGFR signalling but dispensable for degradation. *Dev.Cell.* 15(2): 209-19.