

## Phosphatases and deglycosidases assays for epidermal growth factor receptor (EGFR)

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### INTRODUCTION

EGFR, as many plasma membrane receptors, is heavily glycosylated in the biosynthetic pathway. It also becomes phosphorylated at different Tyrosine and Serine/Threonine residues upon EGF stimulation. This leads to a consistent shift in its molecular weight and to the generation of a smeary pattern in SDS-PAGE gels. This protocol allows the almost complete dephosphorylation and deglycosylation of the receptor in order to reduce the smear at one single sharp band. This allows, for example, a better analysis of the ubiquitination pattern of the EGFR or other proteins of interest.

### MATERIALS

#### 1x JS buffer

- 50 mM HEPES PH 7.5
- mM NaCl
- 1% glycerol
- 1% triton X-100
- 1.5 mM MgCl<sub>2</sub>
- 5 mM EGTA

### METHODS

1. EGFR immunoprecipitates are extensively washed in JS buffer (without phosphatases inhibitors) and subsequently washed once in CIP buffer 1x (Calbiochem).
2. Wash once in CIP buffer 1x (Calbiochem).
3. After the removal of the buffer, the beads are treated for 1 hour at 37°C shaking, with the following phosphatases: Alkaline Calf Intestine Phosphatase and Potato Acid Phosphatase from Calbiochem, Lambda Protein Phosphatase and T-Cell Protein Tyrosine Phosphatase from New England BioLabs, according to the manufacturer's instructions (mixed in CIP buffer 1X, final volume of 50ul).
4. After an additional wash in JS buffer, the IPs were treated for 3 hours at 37°C with a cocktail of deglycosidases (Glycopro Deglycosylation Kit, Prozyme), which includes PNGase F, a non-specific neuraminidase and O-glycosidase, according to the manufacturer's instructions.
5. Finally, the beads are resuspended in 1:1 volume of SDS-PAGE Sample Buffer 2x, boiled 5 minutes at 95°C and loaded on a gel.

### 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.