

Growth Hormone Receptor Pull-down with biotinylated Growth Hormone

Ger Strous, Johan Slotman

University Medical Center Utrecht, The Netherlands

INTRODUCTION

Pull down of growth hormone receptor from cells that express wild type growth hormone receptor. This provides an easy way to study interactors and ubiquitination of the GHR and the factors attached.

MATERIALS

Lysis Buffer

- 1% triton x-100
- 1 mM EDTA
- Protease inhibitors in PBS

Acid Elution Buffer

- 20 mM glycine, pH=2.5

METHODS

1. The required amount of cells are washed 1x with PBS
2. Cells are lysed in lysis buffer (time depends on cell type) and incubated with 100ng biotin-growth hormone (GH) per ml lysate for 1h on ice
3. The lysate is incubated with immobilized streptavidin (pierce) (10 μ l beads/ ml lysate) for 1h at 4°C
4. Beads are spun down (2000 G 10 sec and washed 3 times with lysis buffer and 1 time with 0,1x PBS
5. Elution of GHR from the beads can be done by boiling 3 min in 2xSB. Or for larger volumes in 1 μ l acid elution buffer per μ l beads, this eluate can be concentrated by freeze drying

Alternative for binding GHR in the lysate biotin-gh can be bound to surface receptors only by adding 180ng/ml biotin-GH in HEPES-medium to cells and incubating 1h on ice. Prior to lysis the cells are washed 3x with PBS and then the protocol is followed from point 3.