

Preparation of human LIF

(Hanna lab- by Israel Structural Proteomics Center)

Production:

pGEX-hLIF expression vector was transformed into *E. coli* BL21(DE3) cells which were grown ON in LB-amp medium. Cells were diluted 1:10 into fresh LB-amp medium and grown at 37°C to an OD₆₀₀=0.6 (about 2hr).

IPTG (200 μM final concentration) was added and protein induction was carried out at 15°C (4x1.25L) ON.

Lysis:

Sonication in PBS + 1% TritonX-100

Purification:

1. GST Capture

Add 1mM DTT to clarified lysate and GST beads. Rotate for 2h at 4°C.

2. Remove unbound soup and centrifuge GST-beads through 20% sucrose (layer beads on top of sucrose in PBS and centrifuge at 2500rpm/5min RT. Wash (I) beads with PBS + 1% TritonX-100 and then with 50mM Tris 8,150mM NaCl,1mM CaCl₂ (wash II)).

3. Removal from beads by cleavage with Thrombin:

Add to the beads (in 50mM Tris 8,150mM NaCl,1mM CaCl₂) Thrombine and incubate ON at 4°C.

4. Cation Exchange Chromatography (CEX)

Soup from beads was applied to a Tricorn Mono S 5/50 column which was equilibrated with 20mM Na₂HPO₄ pH 7.2.

The purified hLIF was eluted from the column by applying a gradient of NaCl (elution buffer:20mM Na₂HPO₄, 1M NaCl).