Two-step purification of histidine-tagged membrane proteins using a combination of HisTrap FF crude 1 ml and HiLoad 16/60 Superdex 200 pg

Application

Purification of histidine-tagged membrane proteins by immobilized metal ion affinity chromatography (IMAC) using HisTrap™ FF crude 1 ml column gives high purity for downstream work such as crystallization, especially if an imidazole gradient elution is used. Frequently, however, even higher purity is required. This can be achieved applying a second purification step that removes remaining minor contaminants. The use of a gel filtration column such as HiLoad™ 16/60 Superdex™ 200 pg in the second purification step allows for a fast and generic purification protocol that does not need optimization. This step also ensures removal of imidazole used in the elution buffer, reveals and removes undesirable membrane protein aggregates, and gives a preliminary size estimation of the detergent-protein complex[1].

The main steps in this Procedure are described in Figure 1.

1 Size homogeneity is a useful indicator of stability as membrane proteins tend to oligomerize or aggregate rapidly when destabilized.

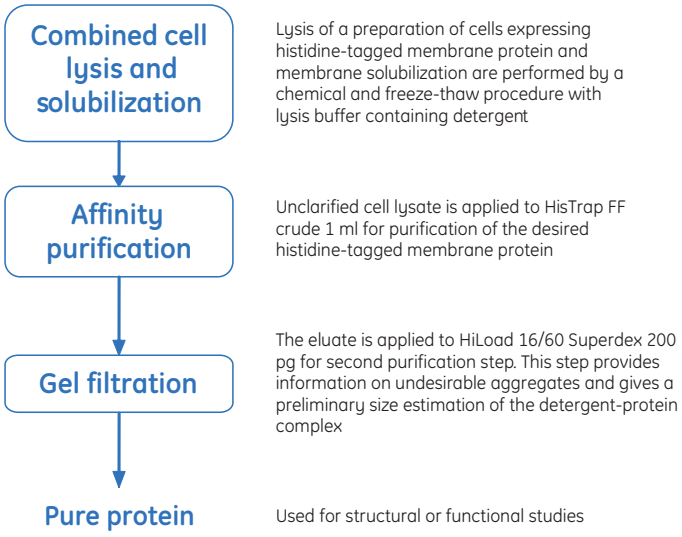


Fig 1. Summary of the purification of membrane proteins using a combination of affinity purification with HisTrap FF crude 1 ml and gel filtration with HiLoad 16/60 Superdex 200 pg.

Cell lysis and membrane protein solubilization

Materials

Solubilization and cell lysis of *E. coli* are achieved by chemical and freeze-thaw lysis using the following buffer:

Lysis buffer: 20 mM sodium phosphate, 100 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 5 U/ml benzonase (nuclease), 1 mg/ml lysozyme, EDTA-free protease inhibitor cocktail, 1% FC12 (detergent), pH 7.4.

Method

Use the following procedure for lysis and solubilization of the membrane protein:

1. Harvest cells from the culture by centrifugation at 8000 × g for 10 min or at 1500 × g for 30 min at 4°C.
2. Discard the supernatant. Place the bacterial pellet on ice.
3. Suspend the bacterial pellet by adding 5 to 10 ml of lysis buffer for each gram of wet cells.
4. Leave for 2 h with mild agitation at room temperature or 4°C, depending on the sensitivity of the target protein.
5. Measure and adjust pH if required.

See reference 1 for further details on cell lysis and solubilization of membrane proteins.

Affinity chromatography of unclarified lysate using HisTrap FF 1 ml column

Materials

Sample: 5 ml unclarified *E. coli* cell lysate with 1% FC12 (detergent)

Binding buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 1% FC12, pH 7.4

Wash buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 40 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% FC12, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 500 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% FC12, pH 7.4.

Purification is performed with HisTrap FF crude 1 ml using a syringe, pump, or chromatography system.

Method

1. Fill the pump tubing or syringe with distilled water. Remove the stopper and connect the column to the chromatography system tubing, syringe (use the adapter provided) or laboratory pump "drop-to-drop" to exclude air from the system.
2. Remove the snap-off end at the column outlet.
3. Remove ethanol storage solution with 3 to 5 ml of distilled water.
4. Equilibrate the column with at least 5 ml of binding buffer. If using a pump or chromatography system, a flow rate of 1 ml/min is recommended
5. Apply 5 ml of unclarified lysate with a pump or a syringe. Continuous stirring of the sample during sample loading is recommended to prevent sedimentation.

   Note: *Large volumes and insufficient lysis can increase back pressure, making the use of a syringe more difficult.*
6. Wash with binding buffer until the UV absorbance measured at 280 nm reaches a steady baseline (generally at least 10 to 15 ml of binding buffer).
7. Elute with elution buffer using a one-step procedure or a linear gradient. For step elution, 5 ml of elution buffer is sufficient.

Further details on the use of this product are found in the Instructions for HisTrap FF crude (code no. 11-0012-38 AE).

Comments

Application of unclarified cell extract reduces manual work and total purification time. Step elution is often satisfactory if used in combination with a second, gel filtration step. Gradient elution, however, allows efficient, automated removal of contaminants that bind to the column.

Figure 2 shows a gradient elution of a histidine-tagged putative transferase protein on HisTrap FF 1 ml connected to an ÄKTAexplorer™ chromatography system. The chromatography conditions described in the "Materials" section were used.

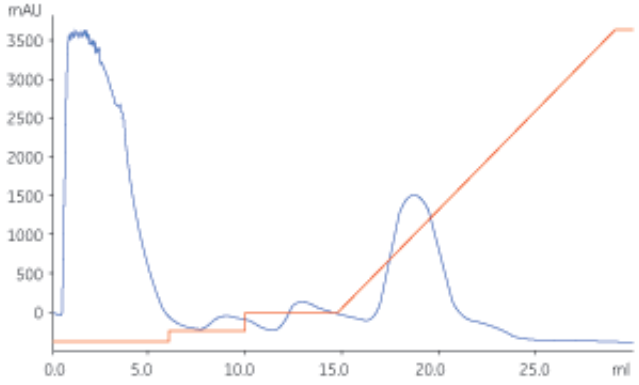


Fig 2. Gradient elution of a histidine-tagged membrane protein on HisTrap FF crude 1 ml.

Gel filtration for final purification of membrane protein

Materials

Sample: Pooled fractions selected from affinity purification step

Equilibration buffer: 20 mM Tris-HCl, 50 mM NaCl, 0.5 mM TCEP, 0.03% DDM, pH 8.0

Method

The target protein fraction (mean eluted peak) is purified in a second step by gel filtration on HiLoad 16/60 Superdex 200 pg column connected to a chromatography system according to the following protocol:

1. Equilibrate the column with 180 to 240 ml of equilibration buffer at a flow rate of 1 ml/min.
2. Apply sample (pooled fractions from affinity purification step).
3. Elute with 120 ml of equilibration buffer, measuring UV absorbance at 280 nm (A280). Collect fractions for further analysis by SDS-PAGE.
4. Before applying a new sample, re-equilibrate column with 120 ml equilibration buffer until the baseline monitored at A280 is stable.

Further details on the use of this product are found in the Instructions for HiLoad 16/60 Superdex 200 pg column (code no. 18-1100-52 AC).

Comments

The gel filtration step removes most of remaining contaminants as well as imidazole used for elution from the HisTrap FF crude column. A major advantage of the Superdex 200 prep grade chromatography medium is its limited interaction with proteins, which allows high recoveries. In addition to purifying the protein, gel filtration provides information on the size of the purified protein and reveals undesired protein aggregates. The data allows comparison with preparations of the same protein in the presence of other detergents or conditions differing in ion strength or pH, and may give indication of what preparation is most suitable for crystallization or other downstream use of the purified protein.

Figure 3 shows gel filtration with HiLoad Superdex 16/60 200 pg after initial affinity purification on HisTrap FF crude.

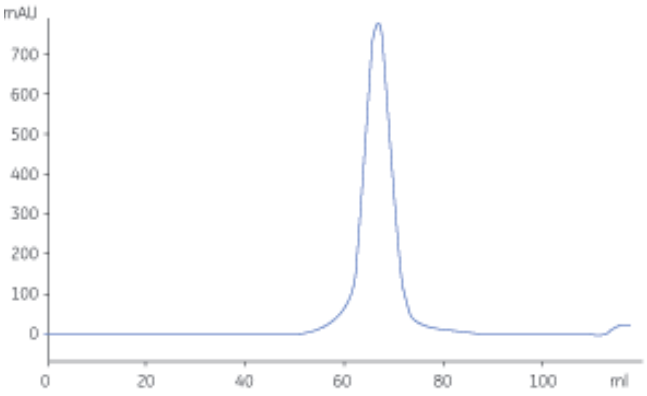
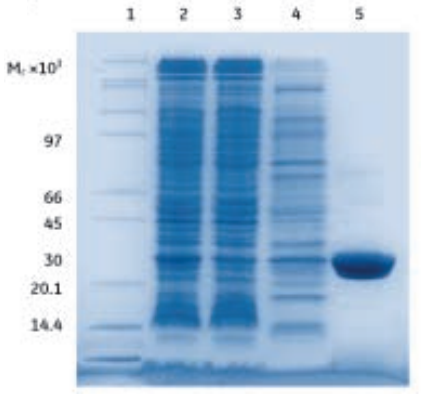


Fig 3. Gel filtration with HiLoad 16/60 Superdex 200 pg allows removal of contaminants as well as aggregates and multimers of a putative transferase membrane protein. Initial purification was performed by affinity chromatography using HisTrap FF crude 1 ml column.

Purity analysis by SDS-PAGE

Standard SDS-PAGE can be used to determine target membrane concentration. Pooled fractions from the peak as analyzed by gel filtration are further analyzed by SDS-PAGE for target protein purity (Fig 4).



Lanes

1. Molecular weight marker proteins
2. Solubilized membranes
3. Flowthrough, HisTrap FF crude 1 ml column
4. Wash, HisTrap FF crude 1 ml column
5. Eluate, HiLoad 16/60 Superdex 200 pg column

Fig 4. SDS-PAGE (Coomassie™ stained gel) of a putative transferase membrane protein after two-step purification using HisTrap FF crude 1 ml and HiLoad 16/60 Superdex 200 pg column. See also reference 2.

References

1. *Purifying Challenging Proteins: Principles and Methods*, GE Healthcare 28-9095-31, Edition AA (2007), pp 15–30. http://www.gelifesciences.com/handbooks
2. Data file: His MultiTrap FF and His MultiTrap HP, GE Healthcare, 11-0036-63, Edition AB (2007).

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