FLAG-Tag Purification

Introduction

Aim: Purifying FLAG-tagged secreted proteins from *E.coli* BL21(DE3).

Timeframe

- 4 hours

Materials

- TBS
    - 50 mM Tris-HCl, 150 mM NaCl, pH 7.4.
- 10 mL FLAG-tag chromatography column from Sigma-Aldrich FLAG® M Purification Kit.
- Wash buffer for FLAG-tagged protein immunoprecipitation kit
- Elution buffer for FLAG-tagged protein immunoprecipitation kit
- 10 ml syringes
- Connector adapter. All buffers from outside the kit should be sterile filtered (0.2 or 0.45 µm) before use.

Procedure

- **Cell Lysis Protocol**

1. Collect the bacterial cell cultures expressing the protein of interest and centrifuge at 5000 x g for 10 minutes.
2. Discard carefully the supernatant and freeze the cell pellet in a -80 ^{o}C freezer.
3. Add CelLytic B 2X at a ratio of 5 mL/g of cell pellet (to extract the maximum amount of protein). In addition, add Lysozyme (to enhance cell lysis) (final concentration 0.2 mg/ml), Benzonase (to decrease viscosity of solution caused by the release of any DNA or RNA) (final amount 50 units/mL) and Protease inhibitor cocktail (to prevent proteases proteolytic action).
4. Incubate the extraction suspension at room temperature for 10-15 minute to fully extract the soluble proteins on rock wave/moving plate.
5. Centrifuge the resulting suspension at maximum rotational speed available in the lab to pellet the insoluble material. Carefully transfer the supernatant into another vial and keep the insoluble material.
6. Resuspend the insoluble material with binding buffer up to the volume prior removal of the supernatant.

7. From both the supernatant and resuspended insoluble material extract 50 µL and add 50 µL of 2x laemmli buffer and keep at – 20 ^{o}C until SDS PAGE is carried out.
8. Mix the supernatant solution with 2X TBS to ensure that the sample is suitable for loading and binding. → In a falcon tube add equal proportions of the supernatant and 2X TBS to form a solution of 1X TBS, 0.15 M NaCl and neutral pH.

- **FLAG Fusion Protein Purification Protocol Using Anti-Flag M2 Affinity Gel**

1. Resin Preparation and Equilibration (at Room Temperature)

    a. Place the empty chromatography column on a firm support.
    b. Rinse the column with TBS twice (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) allowing it to drain and leaving residual TBS to aid the packing of the column.
    c. Prior placing matrix into column, mix gently by inversion the content of the vial to promote even distribution of the resin.
    d. With a pipette transfer the suspension into the column, allow to drain and then rinse the used pipette with TBS, pour content into column and let drain again. (ensures that all the resin sucked by pipette is removed).
    e. Wash the gel by loading three sequential column volumes of 0.1M Glycine-HCl, pH 3.5. (do not disturb gel). Do not leave the column in glycine-HCl for more than 20 minutes.
    f. Wash the resin with 5 CVs of TBS to equilibrate the resin for use. Allow small amount of buffer to stay on top of the column. Do not let gel bed dry.

2. Binding Procedures using Column Chromatography (1 mL of resin will be used).

    a. Add protease inhibitor in elution buffer in correct amounts according to the manufacturer's protocol.
    b. For proper binding of FLAG fusion protein, the sample loaded should be in a buffer containing 0.15 M NaCl and pH 7. (2X TBS & supernatant). Load the sample onto the column under gravity flow and let drain and collect the sample in a Falcon Tube.
    c. From the collected flow-through extract 50 µL in a 1.5 mL Eppendorf and add 50 µL of 2x laemmli buffer and store it in the freezer. The remaining solution will be poured again in the column to enhance the binding efficiency of the packed bed.
    d. Repeat the step 3 times.

3. Washing to Elute any Unbound Proteins.

    a. Wash the column with 15 CVs of TBS and allow the column to drain completely.
    b. Take a sample of 50 µL from one of the wash flow-throughs and place it in a 1.5 mL Eppendorf along with 50 µL of 2x laemmli buffer. Store the sample in the freezer.

4. Elution of Bound FLAG-tagged Proteins by Competition with 3X FLAG Peptide.

    a. Elute by pouring five 1 CV of solution containing 100 µg/mL of 3X FLAG peptide (total of 5 mL hence 500 mg of 3X FLAG peptide) in TBS. Collect 5 elution fragments in 5 sequential 1.5 mL Eppendorf tubes.

5. Recycling the Column.

    a. After elution regenerate the column immediately by washing with 3 CVs of 0.1M Glycine-HCl, pH 3.5. Re- equilibrate the column with TBS until the effluent is at neutral pH.

6. Storing the Column

    a. Wash the column with 10 CVs of 50% glycerol with TBS and 0.02% sodium azide (very toxic DANGER) (100% glycerol and 2X TBS in equal amounts to achieve 50% glycerol solution.) and then add other 5 mL of buffered glycerol (50% glycerol, 10 mM sodium phospahate and 150 mM of NaCl containing 0.02% sodium azide) and store at -20 ^{o} C without draining.

        The column may be reused 3 times before loss of binding capacity is observed.