Murine BPI Production

Bought plasmid from Origene Cat. # MR219864 (more info below)

Transfected into Expi293 cells (using Expi293 Expression system kit Thermo Fisher Cat. # A14635)

Purified using Anti-FLAG Affinity Gel from Bimake Cat. # B23101

DNA purification: (needed more DNA for Expi293 cell transfections) Transfected plasmid into TOP10 comp cells and purified DNA using Qiagen maxi prep kit

Transfection:

1. Warm Expi293 Expression Medium and Opti-MEM I Reduced Serum Medium in water bath.
2. Count cells. Determine size of transfection.
3. Centrfuge cells at 500 RCF at room temperature in 50ml Polypropylene Falcon tubes
4. Resuspend cells at 2.9 x 10^6 cells/ml in fresh Expi293 Expression Medium.
5. In 50ml Polypropylene Falcon tube(s), dilute DNA in Opti-MEM I Reduced Serum Medium. Mix gently by inversion.
6. In a 50ml Polypropylene Falcon tube, dilute ExpiFectamine 293 in Opti-MEM I medium. Mix gently by inversion and incubate for 5 minutes at room temperature.
7. After 5 minutes, add the diluted DNA to the diluted ExpiFectamine 293 Reagent. Mix gently by inverting a couple of times.
8. Incubate the mixture for 20 minutes at room temperature to allow the DNA-Expifectamine 293 Reagent complexes to form.
9. Add dropwise to each flask
10. Incubate cells with shaking (100RPM) at 37°C with 8% CO_2 for 20 hours
11. On Day 1: mix enhancer#1 and enhancer#2 together and then add dropwise
12. Harvest cells on day 5

Purification: Done with BioMT Core Lab

1. Resuspend resin in the vial by inverting or pipetting up and down until the gel is homogeneous.
2. Transfer to a plastic column the appropriate volume of resin and allow the gel bed to drain by gravity. Do not let the gel bed run dry.
3. Equilibrate the column with at least 5 column volumes (CV) of TBS.
4. Load the cell lysate on the column at least 2X to improve capture of the target protein. (did 5X)
5. Collect the flow through (FT) for further testing.
6. Wash the column with 10 CV of TBS buffer. Collect wash fraction (W).
7. Elute the protein using 6X 1 CV of elution buffer. Elute into tubes containing appropriate amount of 1M Tris pH 8.0 to immediately neutralize the eluate (E1 to E6). Elution time cannot exceed 20 min. Immediately regenerate the resin.
8. Resin Regeneration and Storage:
    1. Wash the resin with 5 CV of TBS to raise the pH of the resin.
    2. Wash the resin with 5 CV of 1M NaCl, 5 CV TBS, 5 CV 1% Tween-20, 5 CV TBS
    3. Wash the resin with 5 CV storage buffer. Leave at least 2 mL storage buffer overlaying the resin. Cover with parafilm. Store at 4°C.