SOP: Purification of His-tag GFP using Immobilized Metal Affinity Chromatography

Approvals:

Preparer: Robin M. Zuck Date: 26JUL2018
Reviewer: Dr. Margaret Bryans Date: 08AUG2018

1. **Purpose:** Purify His tagged Enhanced GFP using Immobilized Metal Affinity Chromatography with batch binding and gravity flow.
2. **Scope:**
   2.1. Applies to the purification of recombinant His-tagged GFP from Bacteria cell lysates prepared using B-PER, (bacterial protein extraction reagent).
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Bio Rad Profinity IMAC Resins Instruction Manual
   4.2. Thermo Scientific B-PER Bacterial Protein Extraction Reagent Instructions
5. **Precautions:**
   5.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
   5.2. Gloves and protective eyewear should be worn when handling samples and reagents (buffers).
6. **Materials:**
   6.1. Bio Rad Glass Econo Column 1cm diameter x 5cm long,
   6.2. Bio Rad Profinity IMAC Resin, Nickel charged
   6.3. Clarified DH5α transformed with vector EGFP-pBAD (Addgene Catalog#54762) cell lysate in BPER sample
   6.4. Ring stand and clamp
   6.5. Ice and Ice bucket
   6.6. 250ml beaker filled with metal beads and chilled, for keeping fractions cold during collection.
   6.7. Laboratory balance
   6.8. Tabletop Centrifuge and Rotor
   6.9. Pipettes and tips
   6.10. pH Meter
   6.11. 1.5 ml microtubes and rack for collecting fractions
   6.12. 50ml conical tubes and rack for elution buffers
   6.13. Reagents
      6.13.1. Sodium Phosphate monobasic monohydrate ($NaH_2PO_4 \bullet H_20$)
      6.13.2. Sodium Chloride for molecular biology
      6.13.3. Imidazole
      6.13.4. Milli Q water
      6.13.5. 5M HCl

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6.13.6. 5N NaOH
6.13.7. Protease Inhibitors; Leupeptin, Aprotinin and PMSF stock solutions

7. Procedure:

7.1. **Buffer Preparation**

7.1.1. Buffer A: Binding/Wash buffer, 50mM sodium phosphate, 300mM NaCl, 0mM Imidazole pH8.0, prepare 200ml.
7.1.1.1.Weigh 1.38g of sodium phosphate monobasic monohydrate
7.1.1.2. Weigh 3.51g of sodium chloride
7.1.1.3.Add the sodium phosphate monobasic and the sodium chloride to 150ml MilliQ water
7.1.1.4.Adjust the pH to 8.0, and bring the final volume to 200ml.

7.1.2. Buffer B: Elution Stock Buffer, 50mM sodium phosphate, 300mM NaCl, 500mM Imidazole, pH8.0. Prepare 200ml.
7.1.2.1.Weigh 1.38g of sodium phosphate monobasic monohydrate
7.1.2.2.Weigh 3.51g of sodium chloride
7.1.2.3.Weigh 6.808g of Imidazole
7.1.2.4.Add the sodium phosphate monobasic and the sodium chloride and the Imidazole to 150ml MilliQ water.
7.1.2.5.Adjust the pH to 8.0, and bring the final volume to 200ml.

7.1.3. Elution Gradient Buffers
7.1.3.1. Prepare 10 ml of each of the following buffers by combining Buffer A and Buffer B in 50ml conical tubes;
7.1.3.1.1. 0mM Imidazole: 10ml A
7.1.3.1.2. 25mM Imidazole: 9.5ml of A and 0.5ml of B
7.1.3.1.3. 50mM Imidazole: 9.0ml of A and 1.0ml of B
7.1.3.1.4. 200mM Imidazole: 6.0ml of A and 4.0ml of B
7.1.3.1.5. 300mM Imidazole: 4.0ml of A and 6.0ml of B
7.1.3.1.6. Store these elution buffers at 4°C or in ice for use cold.

7.2. Resin Preparation Note: <u>Do not allow the resin to dry out</u>

7.2.1. Attach a stopcock valve to the bottom of a clean Glass Econo column and close the valve.
7.2.2. Mount the column on the ring stand so that the column is perpendicular and at height that the surface of the resin will be easily viewed and collection tubes and waste container can be positioned under the column outlet. A small beaker containing chilled metal beads can be used to hold the current fraction tube during elution.
7.2.3. Transfer 2ml of the resin slurry to the column, (2ml of slurry is 1ml of resin). Place a waste collection beaker under the column and allow the storage solution to drain into the waste until the meniscus is just above the settled resin. Close the valve. Do not allow the resin to dry out.
7.2.4. Wash the resin with 1 column volume of MilliQ water. (1 column volume, CV, in this case is 1 ml). Carefully add the 1ml of water to the top of the column using a 1000µl pipette and adding the water slowly down the edge of the column being careful not to disturb the

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surface of the resin. Collect the wash in the waste container. Repeat 2 more times for a total of 3 washes.

7.2.5. Equilibrate the resin by washing as above with 5 ml of Binding/Wash Buffer, (Buffer A). Collect the washes in the waste container.

7.2.6. Add 1ml of Binding/Wash buffer to the prepared resin with the valve closed. Gently pipette up and down to resuspend the resin. Transfer the slurry to a 5ml vial with a screw top. Place at 4°C or on ice for use cold.

7.3. Sample Preparation

7.3.1. Preparation of bacterial cell lysate;

7.3.1.1. Weigh an empty 1.5 ml microfuge tube and record the weight.

7.3.1.2. Transfer approximately 100 mg of bacterial cell pellet to the pre-weighed microfuge tube and determine the weight of cell pellet transferred.

7.3.1.3. Resuspend the pellet in a 1:10 ratio (w/v) of B-PER, (Bacterial Protein Extraction Reagent) by pipetting up and down. Use 1000µl for a 100mg of cell pellet.

7.3.1.4. Protease inhibitors can be added to this lysate if desired;

7.3.1.4.1. Add 2.5µl of a 0.2mg/ml solution of Leupeptin in MilliQ water.

7.3.1.4.2. Add 2µl of a 1.0 mg/ml solution of Aprotinin in MilliQ water.

7.3.1.4.3. Add 4µl of a 10mg/ml solution of PMSF in isopropanol.

7.3.1.5. Vortex for 10 seconds.

7.3.1.6. Shake for 10 minutes at room temperature on a multi wrist shaker.

7.3.1.7. Centrifuge the lysate at 15,000 rpm for 10 minutes at room temperature.

7.3.2. Transfer the supernatant to a fresh microtube

7.3.3. If the lysate is not clear, filter the lysate using a 0.2µm syringe filter.

7.3.4. Reserve 30µl of clarified lysate for later analysis.

7.4. Binding

7.4.1. Add up to 1ml of clarified lysate to the prepared resin slurry in the 5ml vial.

7.4.2. Incubate the lysate-resin mixture at 4°C or on ice with gentle mixing for 30 minutes. Place the closed vial flat in the ice and rock a couple of times every 3 to 5 minutes.

7.5. During this incubation label 1.5ml microtubes for the fractions to be collected as follows; FT/1, FT/2, 25/1, 25/2, 50/1, 200/1, 200/2, 200/3 and 300/1.

7.6. Elution

7.6.1. Keep elution buffers and collected fractions on ice. Transfer the lysate-resin mixture to the column, be sure the column valve is closed. Allow the resin to settle in the column.

7.6.2. Collect the Flow Through into the labeled microtubes being careful not to let the resin dry out. Close the column valve.

7.6.3. Add 1 CV of 25mM Imidazole buffer slowly to the column allowing the buffer to run down the side of the column and not disrupt the surface of the resin. Once added collect this fraction in the 25/1 labelled tube. Close the valve.

7.6.4. Repeat this process again using the 25mM Imidazole buffer, (fraction 25/2).

7.6.5. Add 1 CV of the 50mM Imidazole buffer and collect the fraction in the labeled tube.

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7.6.6. Elute 3 times with 1 CV each of the 200mM Imidazole buffer and collect the fractions, (200/1, 200/2, 200/3). These fractions should contain the purified GFP. If there is GFP visible in the 200/3 fraction collect another 200mM Imidazole fraction, 200/4.

7.6.7. Wash the column once with 1 CV of the 300mM Imidazole buffer. Confirm that there is no GFP in the 300mM fraction.

7.6.8. Save the collected fractions at 4°C for further analysis.

7.6.9. The presence or absence of GFP in the fractions can be visualized by viewing the tubes on the blacklight table. The 300mM Imidazole fraction should contain no or very little GFP. If there appears to be substantial GFP in this fraction wash the column with another CV of 300mM Imidazole buffer and save the fraction.

7.6.10. Regenerate the resin

7.6.10.1. Wash the column with 5 CVs of the 500mM Imidazole buffer and collect the wash in a waste container.

7.6.10.2. Wash the column with 5CVs of the 0mM Imidazole buffer and collect the wash in the waste container.

8. History:

Revision Number	Effective Date	Preparer	Description of change
0	08/08/2018	Robin Zuck	Initial release