Workflow of the Manual Purification of N/NC5-enriched proteins

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Criteria for manual purification

• If the extinction coefficient is less than 4000.
• If the protein had previously failed purification in the AKTA Express
Day One
Two Step Purification

Day Two
SDS-PAGE of fractions collected by AKTA

Day Three
Pool desired fractions and Concentrate

Day Four
Buffer Exchange and Concentrate

Day Five
Final SDS-PAGE and Mass Spec

Day Six
Bulk Upload and NMR Sample Prep

Outline
Day One

1. Obtain necessary info from Spine for each protein

2. Get pellet from freezer

3. Resuspend pellet in 25 ml of Lysis Buffer

4. Sonicate cell suspension (Total)

5. Obtain supernatant by centrifugation

6. Filter supernatant (0.45μm) (Soluble)

7. Prepare Ni-NTA Superflow column

8. Pour the supernatant into The Ni-NTA Superflow Column (F.T.)

9. Wash the Column with 50ml of Wash Buffer (W)

10. Elute the protein from the Ni-NTA column using 10ml of Elution Buffer (E)

11. Run an SDS PAGE Gel Of the Total, Soluble, Flow through, Wash and Elution Samples

12. Inject the Samples into the AKTA Express and let them run overnight.
Day Two

Analyze chromatograph and decide which fractions to run for SDS-PAGE

Decide which fractions to pool based on result of chromatograph and SDS-PAGE

MAINTAIN THE AKTA
Day Three

Pool fractions Based Unicorn Result

Concentrate Sample to 0.5-1.0 mM By Amicon Ultrafree Device

Determine Concentration at 280nm by diluting protein with 6M Guanadine + 10mM Tris, pH 7.5

In case of precipitation
Stop further concentrating, remove precipitate by centrifugation and Analyze supernatant
Day Four

- Buffer exchange into selected NMR Buffers using desalting columns if there is enough protein.
- Concentrate Sample to 0.5-1.0 mM by Amicon Ultrafree Device

Bulk Purification Uploads into SPiNE – Purification Template

<table>
<thead>
<tr>
<th>Expression ID</th>
<th>New entry</th>
<th>Researcher</th>
<th>Date Purified</th>
<th>Method</th>
<th>Purification Buffer</th>
<th>final buffer</th>
<th>final conc</th>
<th>final vol</th>
<th>Oligomerization State</th>
<th>Determined by</th>
<th># Tubes</th>
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</thead>
<tbody>
<tr>
<td>SR361.21-NC5a</td>
<td>NI</td>
<td>Kanishma Shetty, Rong Xiao</td>
<td>9/28/2005</td>
<td>Ni-NTA</td>
<td>Binding Buffer</td>
<td>Elution Buffer</td>
<td>0.03</td>
<td>10</td>
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<td>0</td>
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<td>SR361-21.2-NC5a</td>
<td>GF</td>
<td>Kanishma Shetty, Rong Xiao</td>
<td>9/29/2005</td>
<td>Superdex75</td>
<td>Binding Buffer</td>
<td>NMR Buffer 8.5</td>
<td>1.53</td>
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<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Proteins with MW with a deviation greater than 500 Daltons from the reported value in SPiNE are held and submitted for LC-MS analysis (PeterLobel’s group).
Day Six

- SDS-PAGE pictures taken
- Information Placed in SPiNs In JPEG format
- Information Uploaded onto SPiNE
- Protein information uploaded into SPiNS from SPiNE.
- NMR Samples Made, placed in the inbox and email sent out (Swapna, Tom and Rong)
- Upload PST Information into SPiNE
Purification Flowchart

Proteins attempted to be purified

Failed purification

- 42 Proteins (28.6%)

  - \( E \times S \leq 4 \)
    - 32 Proteins (76.2%)
    - No Binding or Aggregates in GF
      - Change the buffer & purify
      - Manually or make a new Construct.
      - NOTE: Test for hex-his tag.

  - \( E \times S > 4 \)
    - 10 Proteins (23.8%)

Successfully purified

- 105 Proteins (71.4%)
Proteins attempted to be purified

147 Proteins

Failed purification

42 Proteins (28.6%)

E x S <= 8

35 Proteins (83.33%)

No Binding or Aggregates in GF

Change the buffer & purify manually or make a new construct.
NOTE: Test for hex-his tag.

E x S > 8

7 Proteins (16.67%)

Successfully purified

105 Proteins (71.4%)