Objectives
Clean used GST resin so that it may be used in future protein purifications.

Materials
- GST Resin
- 70% Ethanol
- GST Elution Buffer

Equipment
- Centrifuge
- Vortexer
- Micropipette
- Eppendorf tubes/Falcon tubes
- Spectrophotometer
- Cuvettes
Procedure

☐ **Step #1:** Dispense dirty GST resin/buffer solution into eppendorf tubes or a falcon tube.

☐ **Step #2:** Centrifuge tubes @ 4.0°C ~1000 RPM for 2 minutes (or until a firm pellet is formed and supernatant looks fairly clear). Discard supernatant.

☐ **Step #3:** Add 3 “bed volumes” of 70% Ethanol to each tube and vortex gently to mix.

☐ **Step #4:** Centrifuge tubes @ 4.0°C ~1000 RPM for 2 minutes (or until a firm pellet is formed and supernatant looks fairly clear). Discard supernatant.

☐ **Step #5:** If multiple eppendorf tubes were used, combine all resin samples into 1 falcon tube and add 3 “bed volumes” of GST Elution Buffer. Pipette 1.0 mL of GST Elution Buffer into a cuvette and label “Blank.”

☐ **Step #6:** Place tube in end over end rocker or table top rotator for 30.0 minutes.

0:30:00

☐ **Step #7:** Dispense resin/buffer solution into multiple eppendorf tubes or a falcon tube and centrifuge tube(s) @ 4.0°C ~1000 RPM for 2 minutes (or until a firm pellet is formed and supernatant looks fairly clear).

☐ **Step #8:** Collect a 1.0 mL sample of the supernatant and dispense into a cuvette labeled “E1.” Discard the rest of the supernatant.

☐ **Step #9:** Blank a spectrophotometer set to OD280 with the clean GST Elution Buffer.

☐ **Step #10:** Measure the absorbance of sample E1 at 280 nm. If the reading is not atleast 0.0*** then repeat steps 7-10.

☐ **Step #11:** After the correct 280A is reached, resuspend the resin in enough 70%
Ethanol solution to achieve a 50/50 (resin/ETOH) mixture.

Step #12: Store cleaned resin @ 4.0°C until needed.

Recipes

70% Ethanol Solution
- 14.0 mL 100% Ethanol
- 6.0 mL diH2O

GST Elution Buffer
Yields 30.0 mL
- 50 mM Tris (121.14 g/mol)
  - 302.68 mg
- 25 mM Reduced Glutathione (307.3 g/mol)
  - 383.85 mg
- 200 mM NaCl (58.44 g/mol)
  - 579.59 mg
- ~30.0 mL diH2O

References

Elution buffer recipe obtained from: http://labs.mmg.pitt.edu/gjoerup/GST%20fusions-%20elution2.doc

GST Resin and protocol instructions obtained from Grad Student advisor Jessica Siemer

Results

Sample absorbances @280 nM using Implen 300 Spectrophotometer

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance @ 280 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean GST Elution Buffer &quot;Blank&quot;</td>
<td>0</td>
</tr>
<tr>
<td>Elution 1</td>
<td>0.667</td>
</tr>
<tr>
<td>Elution 2</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Conclusion

Will you publish these results?