Materials List for:
Affinity Precipitation of Active Rho-GEFs Using a GST-tagged Mutant Rho Protein (GST-RhoA(G17A)) from Epithelial Cell Lysates

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### Materials

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGEX-RhoA(G17A)</td>
<td>Addgene</td>
<td>Will be available soon</td>
<td>Generated in the lab of Dr. Keith Burridge (University of North Carolina) (ref 3)</td>
</tr>
<tr>
<td>DH5α</td>
<td>Invitrogen</td>
<td>18258-012</td>
<td>Store -80°C</td>
</tr>
<tr>
<td>SOC medium</td>
<td>BioShop Canada</td>
<td>SOC001.10</td>
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</tr>
<tr>
<td>LB</td>
<td>BioShop Canada</td>
<td>LBL407.1</td>
<td>Keep sterile</td>
</tr>
<tr>
<td>Glycerol</td>
<td>BioShop Canada</td>
<td>GLY002.1</td>
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<tr>
<td>Ampicillin</td>
<td>BioShop Canada</td>
<td>AMP201.25</td>
<td>Store stock at -20°C</td>
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<tr>
<td>Agar</td>
<td>BioShop Canada</td>
<td>AGR001.500</td>
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</tr>
<tr>
<td>IPTG (Isopropyl B-D-thiogalactopyranoside)</td>
<td>Calbiochem</td>
<td>420322</td>
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<tr>
<td>Glutathione Sepharose beads</td>
<td>GE Healthcare</td>
<td>17-0756-01</td>
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</tr>
<tr>
<td>PBS 10x</td>
<td>Sigma-Aldrich</td>
<td>D1408</td>
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<tr>
<td>Hapes</td>
<td>Sigma-Aldrich</td>
<td>H4034</td>
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<td>NaCl</td>
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<tr>
<td>MgCl₂</td>
<td>Sigma-Aldrich</td>
<td>M-9272</td>
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<td>Sigma-Aldrich</td>
<td>X100</td>
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<tr>
<td>DTT</td>
<td>OmniPur, EMD Millipore</td>
<td>3860</td>
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<td>P-7626</td>
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<td>Protease Inhibitor 50x</td>
<td>BD Biosciences</td>
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<td>Complete Mini, EDTA-free 10x</td>
<td>Roche Group</td>
<td>11 836 170 001</td>
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<tr>
<td>Laemmli Sample Buffer 2x</td>
<td>Bio-Rad</td>
<td>161-0737</td>
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<tr>
<td>β-mercapt–thanol</td>
<td>Sigma-Aldrich</td>
<td>M7154</td>
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<td>BLUEye ladder</td>
<td>FroggaBio</td>
<td>PM008-0500</td>
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</tbody>
</table>

Table 1. Reagents used

<p>| RC-5B centrifuge                  | Sorvall, Thermo Scientific | SS-34 Rotor | Use at 4°C                                   |
| Centrifuge                        | Sorvall, Thermo Scientific | ST-16R      | Use at 4°C                                   |
| Micro centrifuge                  | Eppendorf            | 5417R       | Use at 4°C                                   |
| Bacterial shaker                  | INFORS HT Ecotron    | AG CH-1403 Bottmingen | Use at 37°C or at 22°C                       |</p>
<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
<th>Model</th>
<th>Temperature</th>
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</thead>
<tbody>
<tr>
<td>Sonicator</td>
<td>Branson</td>
<td>Sonifier-450</td>
<td>Use at RT</td>
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<tr>
<td>Rotator</td>
<td>Glas-Col</td>
<td>099A CR4012</td>
<td>Use at 4°C</td>
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*Table 2. Specific equipments used*