

Materials List for:

Identification of Inositol Phosphate or Phosphoinositide Interacting Proteins by Affinity Chromatography Coupled to Western Blot or Mass Spectrometry

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Materials

| Name | Company | Catalog Number | Comments |
|--|-------------------------|----------------|--|
| Acetone | Sigma-Aldrich | 650501 | Ketone |
| Acetonitrile | Sigma-Aldrich | 271004 | Solvent |
| Ammonium bicarbonate | Sigma-Aldrich | A6141 | Inorganic salt |
| Centrifuge Avanti J6-MI | Beckman Coulter | Avanti J6-MI | Centrifuge for large volumes (e.g., 1L) |
| Centrifuge bottles | Sigma-Aldrich | B1408 | Bottles for centrifugation of 1L of culture |
| Control Beads | Echelon | P-B000-1ml | Affinity chromatography reagent - control |
| D-(+)-Glucose | Sigma-Aldrich | G8270 | Sugar, Added in PBS to keep cells viable |
| Dithiothreitol (DTT) | Bio-Rad | 1610610 | Reducing agent |
| Dynabeads M-270 Streptavidin | ThermoFisher Scientific | 65305 | Streptavidin beads for binding to biotin ligands |
| EDTA-free Protease Inhibitor Cocktail | Roche | 11836170001 | Protease inhibitors |
| Electrophoresis running buffer | Bio-Rad | 1610732 | 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3 |
| Falcon 15 mL Conical Centrifuge Tubes | Corning Life Sciences | 430052 | To centrifuge 10 mL cultures |
| Formic acid | Sigma-Aldrich | 106526 | Acid |
| Glycine | Sigma-Aldrich | G7126 | Amino acid |
| HMI-9 cell culture medium | ThermoFisher Scientific | ME110145P1 | Cell culture medium for <i>T. brucei</i> bloodstream forms |
| Imperial Protein Stain | ThermoFisher Scientific | 24615 | Coomassie staining for protein detection in SDS/PAGE |
| Ins(1,4,5)P3 Beads | Echelon | Q-B0145-1ml | Affinity chromatography reagent |
| Instant Nonfat Dry Milk | Thomas Scientific | C837M64 | Blocking reagent for Western blotting |
| Iodoacetamide | Sigma-Aldrich | I6125 | Alkylating reagent for cysteine proteins or peptides |
| Lab Rotator | Thomas Scientific | 1159Z92 | For binding assays |
| LoBind Microcentrifuge Tubes | ThermoFisher Scientific | 13-698-793 | Low protein binding tubes for mass spectrometry |
| Nonidet P-40 (Igepal CA-630) | Sigma-Aldrich | 21-3277 | Detergent |
| PBS, pH 7.4 | ThermoFisher Scientific | 10010031 | Physiological buffer |
| Peroxidase substrate for chemiluminescence | ThermoFisher Scientific | 32106 | Substrate for Western blotting detection of proteins |

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| PhosSTOP Phosphatase Inhibitor Cocktail Tablets | Roche | 4906845001 | Phosphatase inhibitors |
| PI(3)P PIP Beads | Echelon | P-B003a-1ml | Affinity chromatography reagent |
| PI(3,4)P2 PIP Beads | Echelon | P-B034a-1ml | Affinity chromatography reagent |
| PI(3,4,5)P3 diC8 | Echelon | P-3908-1mg | Affinity chromatography reagent |
| PI(3,4,5)P3 PIP Beads | Echelon | P-B345a-1ml | Affinity chromatography reagent |
| PI(3,5)P2 PIP Beads | Echelon | P-B035a-1ml | Affinity chromatography reagent |
| PI(4)P PIP Beads | Echelon | P-B004a-1ml | Affinity chromatography reagent |
| PI(4,5)P2 diC8 | Echelon | P-4508-1mg | Affinity chromatography reagent |
| PI(4,5)P2 PIP Beads | Echelon | P-B045a-1ml | Affinity chromatography reagent |
| PI(5)P PIP Beads | Echelon | P-B005a-1ml | Affinity chromatography reagent |
| Ponceau S solution | Sigma-Aldrich | P7170 | Protein staining (0.1% [w/v] in 5% acetic acid) |
| Potassium hexacyanoferrate(III) | Sigma-Aldrich | 702587 | Potassium salt |
| PtdIns PIP Beads | Echelon | P-B001-1ml | Affinity chromatography reagent |
| PVDF Membrane | Bio-Rad | 1620177 | For Western blotting |
| Refrigerated centrifuge | Eppendorf | 5910 R | Microcentrifuge for small volumes (e.g., 1.5 mL) |
| Sodium dodecyl sulfate | Sigma-Aldrich | 862010 | Detergent |
| Sodium thiosulfate | Sigma-Aldrich | 72049 | Chemical |
| SpeedVac Vacuum Concentrators | ThermoFisher Scientific | SPD120-115 | Sample concentration (e.g., for mass spectrometry) |
| T175 flasks for cell culture | ThermoFisher Scientific | 159910 | To grow 50 mL <i>T. brucei</i> culture |
| Trypsin, Mass Spectrometry Grade | Promega | V5280 | Trypsin for protein digestion |
| Urea | Sigma-Aldrich | U5128 | Denaturing reagent |
| Vortex | Fisher Scientific | 02-215-418 | For mixing reactions |
| Western blotting transfer buffer | Bio-Rad | 1610734 | 25 mM Tris, 192 mM glycine, pH 8.3 with 20% methanol |
| Whatman 3 mm paper | Sigma-Aldrich | WHA3030861 | Paper for Wester transfer |
| 2-mercaptoethanol (14.2 M) | Bio-Rad | 1610710 | Reducing agent |
| 2x Laemmli Sample Buffer | Bio-Rad | 161-0737 | Protein loading buffer |
| 4–20% Mini-PROTEAN TGX Precast Protein Gels | Bio-Rad | 4561094 | Gel for protein electrophoresis |
| 4x Laemmli Sample Buffer | Bio-Rad | 161-0747 | Protein loading buffer |