

Materials List for:

Isolation of Cellular Lipid Droplets: Two Purification Techniques Starting from Yeast Cells and Human Placentas

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Materials

| Name | Company | Catalog Number | Comments |
|--|--------------------------|----------------|---|
| Protocol 1 | | | |
| 1. Growing yeast cells and converting to spheroplasts | | | |
| Edinburgh Minimal Media (EMM) | Sunrise Science Products | 2005 | |
| Yeast extract with 5 supplements (YE5S) | Sunrise Science Products | 2011 | YE5S media with 225 mg/ml of each supplement: adenine, histidine, leucine, lysine, uracil. The equivalent for budding yeast would be YPD. |
| YPD powder | Sunrise Science Products | 1875 | For <i>S. cerevisiae</i> |
| Sorbitol | Fisher Scientific | BP439 | |
| Yeast Lytic Enzyme | MP Biomedicals | 215352610 | |
| Lysing Enzymes from <i>Trichoderma harzianum</i> | Sigma-Aldrich | L1412 | |
| Zymolayse-20T | Sunrise Science Products | N0766391 | For <i>S. cerevisiae</i> |
| BODIPY 493/503 | Invitrogen | D-3922 | |
| Microscope Slides | Fisher Scientific | 12-544-7 | |
| Microscope Cover Glass | Fisher Scientific | 12-542-B | |
| Plastic transfer pipette | Fisher Scientific | 137115AM | |
| 1 L glass bottle | | | |
| 250 ml flask | | | |
| 2.8 L flasks | | | |
| 2. Yeast lipid droplet isolation | | | |
| Tris-HCl | Fisher Scientific | BP153 | |
| EDTA | Fisher Scientific | BP120 | |
| Ficoll 400 | Fisher Scientific | BP525 | |
| 12-14k Spectra/Por Dialysis Membrane | SpectrumLabs | 132680 | |
| EDTA-free Protease Inhibitor Cocktail Tablets | Roche Diagnostics | 11873580001 | irritant |
| Dounce Homogenizer | Sigma-Aldrich | D9938 | |
| Ultracentrifuge Tubes 25 mm x 89 mm (for SW28) | Beckman-Coulter | 355642 | |
| 12-14k Spectra/Por Dialysis Membrane | SpectrumLabs | 132680 | |

| | | | |
|---|--------------------------|-------------|----------|
| Temperature-controlled shaker | New Brunswick Scientific | C25KC | |
| Thermo Sorvall Legend XTR centrifuge | Thermo-Scientific | 75004521 | |
| Swinging Bucket Centrifuge Rotor | Thermo-Scientific | 75003607 | |
| Fiberlite* F15-6x100y Fixed-Angle Rotor | Thermo-Scientific | 75003698 | |
| Ultracentrifuge LB-M | Beckman-Coulter | | |
| SW28 Ultracentrifuge Rotor | Beckman-Coulter | 342204 | |
| Protocol 2 | | | |
| 1. Placental villous cells isolation | | | |
| Disposable underpads | Fisher Scientific | 23666062 | |
| Autoclavable pan (container), 3 L | Fisher Scientific | 1336110 | |
| Fine scissors, sharp-sharp, straight | Fine Science Tools | 1406011 | |
| London Forceps | Fine Science Tools | 1108002 | |
| Dumont #7b Forceps | Fine Science Tools | 1127020 | |
| Razor blades | Fisher Scientific | S65921 | |
| Screen cup for CD-1 | Fisher Scientific | S1145 | |
| 40 mesh screen | Fisher Scientific | S0770 | |
| Fisherbrand cell stainers 100 µm | Fisher Scientific | 22363549 | |
| 150 mm Petri Dishes | Fisher Scientific | NC9054771 | |
| NaCl | Fisher Scientific | S642 | |
| KCl | Fisher Scientific | P333 | |
| KH ₂ PO ₄ | Fisher Scientific | P386 | |
| Na ₂ HPO ₄ | Fisher Scientific | S374 | |
| D-Glucose | Fisher Scientific | D16 | |
| HEPES | Fisher Scientific | BP310 | |
| 2.5% Trypsin 10x | Invitrogen | 15090046 | |
| DNase I grade II, from bovine pancreas | Roche Applied Science | 10104159001 | |
| Sodium bicarbonate solution | Sigma-Aldrich | S8761 | |
| 500 ml Erlenmeyer flasks | | | |
| 250 ml Beakers | | | |
| 15 ml Centrifuge tubes | | | |
| 10 ml Serological pipettes | | | |
| 50 ml Centrifuge tubes | | | |
| DMEM | Invitrogen | 11965084 | |
| 2. Lipid droplets isolation from villous placental cells | | | |
| Tris-HCl | Fisher Scientific | BP153 | |
| EDTA | Fisher Scientific | BP120 | |
| D-Sucrose | Fisher Scientific | BP220 | |
| Sodium Carbonate | Fisher Scientific | BP357 | |
| EDTA-free protease inhibitor cocktail tablets | Roche Diagnostics | 11873580001 | irritant |
| Dounce homogenizer | Sigma-Aldrich | D9938 | |
| Ultracentrifuge tubes 25 mm x 89 mm (for SW28) | Beckman-Coulter | 355642 | |

| | | | |
|---|---------------------------|--|-------------------|
| Ultra-Clear centrifuge tubes 14 mm x 89 mm (for SW41) | Beckman-Coulter | 344059 | |
| Disposable borosilicate glass Pasteur pipets | Fisher Scientific | 1367820C | |
| Biological safety hood | Thermo-Scientific | | |
| Waterbath | Fisher Scientific | | |
| Temperature-controlled shaker | New Brunswick Scientific | C25KC | |
| Thermo Sorvall Legend XTR centrifuge | Thermo-Scientific | 75004521 | |
| Swinging Bucket Centrifuge Rotor | Thermo-Scientific | 75003607 | |
| Ultracentrifuge LB-M | Beckman-Coulter | | |
| SW28 Ultracentrifuge Rotor | Beckman-Coulter | 342204 | |
| SW41 Ti Ultracentrifuge Rotor | Beckman-Coulter | 331336 | |
| Western blot | | | |
| IRDye 680 Goat Anti-Rabbit IgG | LI-COR | 926-68071 | dilution 1:15,000 |
| IRDye 800CW Goat Anti-Mouse IgG | LI-COR | 926-32210 | dilution 1:5,000 |
| NuPAGE Novex 12% Bis-Tris gels | Invitrogen | NP0341 | |
| Primary antibodies for Protocol 1 | | | |
| Erg6p | gift from Dr. G. Daum | Graz University of Technology, Austria | dilution 1:5,000 |
| Dpm1p | Abcam | ab113686 | 4 µg/ml |
| Por1p | gift from Dr. G. Daum | Graz University of Technology, Austria | dilution 1:5,000 |
| Pma1p | gift from Dr. G. Daum | Graz University of Technology, Austria | dilution 1:10,000 |
| Vma1p (anti-ATP6V1A) | Abcam | ab113745 | 0.5 µg/ml |
| Primary antibodies for Protocol 2 | | | |
| Perilipin 2 (anti-ADFP) | Abcam | ab52355 | 2 µg/ml |
| Calnexin | Cell Signaling Technology | 2679 | dilution 1:1,000 |
| GM130 | Biorbyt | orb40533 | dilution 1:25 |
| COX IV | Cell Signaling Technology | 4850 | dilution 1:1,000 |
| MEK1 | Biorbyt | orb38775 | dilution 1:50 |