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Preparation of Cell Culture Samples for Cytokine Analysis (with shipping instructions)

Follow the protocols below for adherent and suspension cells respectively. Please read the manual accompanying the Cell Lysis Buffer carefully. Volume of Procarta Cell Lysis Buffer should be scaled to obtain at least 3 micrograms of protein per microliter of lysate.

1. Remove media from cells. Reserve and freeze this at -80°C if you wish to analyze secreted proteins.

2a. **Adherent Cells**
Preparation is for 5x10^6 to 2x10^7 cells grown in a 10 cm plate. Trypinsinze the cells and remove the cells using a cell scraper. Transfer the cells to a 15 mL conical tube and centrifuge at 500 x g at 4° C for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 mL of ice cold PBS. Repeat this step using 5 ml of ice cold PBS and centrifuge again at 500 x g at 4° C. Completely remove all of the PBS.

2b. **Suspension Cells**
Preparation is for 5x10^6 to 2x10^7 cells grown in a flask. Transfer cells to a 15 mL conical tube and spin at 500 x g at 4° C for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 ml of ice cold PBS and spin again at 500 x g at 4° C. Completely remove all of the PBS.

3. Add ice cold Procarta Cell Lysis Buffer Panomics (Affymetrix CAT#: EPX-99999) supplemented with 1mM PMSF (final concentration) and 1X protease inhibitor cocktail (Sigma-Aldrich CAT #: P8340), pipette up and down several times and then incubate on ice for 5 minutes. Please list concentrations of buffer components on sample submission sheet.

4. Transfer entire contents to a 1.5 mL microcentrifuge tube

5. Centrifuge at 14,000 rpm (bench top centrifuge) for 10 minutes at 4° C.

6. Transfer the supernatant to a new tube and store the samples at -80°C.

Shipping

Select a box for shipment that has an external dimension of at least 25 cm in each dimension. The box should have a sturdy cardboard exterior and inner Styrofoam box with wall thickness of at least 2.5 cm. Fill the storage box with 1.5 kg of dry ice. Cool a 133 mm x 133 mm x 48 mm (2 ml tubes) or 73 mm (5 ml tubes) vial storage box in the shipping container.[3] Transfer the frozen sample tubes to the vial storage box. Fill in the free areas of the vial storage box with dry ice and secure the vial storage box top over the samples using wire or string. Fill the Styrofoam shipping container with additional dry ice, and packing peanuts (if necessary) in order to minimize free space in the package. The total dry ice content should be at least 2.5 kg. Use FedEx overnight delivery to ship the package to ORB at the address below. Affix the dry ice sticker to the exterior of the package and record the same dry ice weight on the sticker as was used during set-up of the shipment (may require metric to English unit conversion).

Attention:
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