



## OCEAN RIDGE BIOSCIENCES

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### Preparing Cells for Cytokine Profiling on Luminex System

1. Remove media from cells. Reserve and freeze this at  $-80^{\circ}\text{C}$  if you wish to analyze secreted proteins.
2.
  - a. *Adherent Cells*  
Preparation is for  $5 \times 10^6$  to  $2 \times 10^7$  cells grown in a 10 cm plate. Trypsinize the cells and remove the cells using a cell scraper. Transfer the cells to a 15 ml conical tube and centrifuge at  $500 \times g$  at  $4^{\circ}\text{C}$  for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 mls of ice cold PBS. Repeat this step using 5 ml of ice cold PBS and centrifuge again at  $500 \times g$  at  $4^{\circ}\text{C}$ . Completely remove all of the PBS.
  - b. *Suspension Cells*  
Preparation is for  $5 \times 10^6$  to  $2 \times 10^7$  cells grown in a flask. Transfer cells to a 15 ml conical tube and spin at  $500 \times g$  at  $4^{\circ}\text{C}$  for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 ml of ice cold PBS and spin again at  $500 \times g$  at  $4^{\circ}\text{C}$ . Completely remove all of the PBS.
3. Add 250  $\mu\text{L}$  of ice cold Procarta Cell Lysis Buffer Panomics/ Affymetrix Part # PC6002) containing protease inhibitors, 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  $4^{\circ}\text{C}$ .
6. Transfer the supernatant to a new tube and store the samples at  $-80^{\circ}\text{C}$ .

### Sample Requirements for Profiling of Cell Lysates on Luminex System

- For optimal results please provide 225  $\mu\text{l}$  of cleared lysate at a protein concentration of 5-10  $\mu\text{g}/\mu\text{l}$ .
- The minimum amount of sample that would provide useful results is 75  $\mu\text{l}$  of lysate at a concentration of  $>3 \mu\text{g}/\mu\text{l}$ .

### Sample Requirements for Profiling of Cell Media on Luminex System

- Provide at least 225  $\mu\text{l}$  of conditioned media.