Whole Cell Lysate from cells

Protein Harvesting

Mix lysis buffer

Ripa Buffer +100x PI cocktail + 100x Na3VO4

For 6well plate, need 150ul Lysis buffer/well

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|  | 1 well | 1 well\* | ­ wells |
| RIPA Buffer | 150ul | 25uL | 25uL x ­ wells = . |
| PI cocktail (100x) | 1.5ul | 0.25uL | 0.25uL x ­ wells = . |
| Na3VO4 (100x) | 1.5ul | 0.25uL | 0.25uL x ­ wells = . |

\*For more concentrated samples

* Place cell plates on ice
* Prepare Ripa buffer with PI cocktail and Na3VO4 (see above)
* Remove media from wells
* Wash with 2ml/well PBS and aspirate completely, tilting plate to ensure that all PBS is off the plate
* Add150uL or **25**uL Lysis buffer/well
* Rock for 1-10min at 4°C
* Scrape cells and transfer to epi tubes
* Incubate on ice 30min, occasionally vortexing
* Centrifuge 10min @10,000rpm @4°C
* Remove supernatant to fresh tube. This is whole cell lysate
* WCL should be stored at -80°C