Protocol for Lysis of Small Cell Pellets (without protein concentration assay)

This protocol is usually used for special projects with limited number of cells. A pilot project is recommended to test the volume required to generate acceptable protein concentration. Please consult core director/staff before using this protocol.

Reagents provided by the Core:

3 ml RPPA Lysis Buffer (store at 4°C)
1 ml 5X Protease Inhibitors (store at -20°C, use by expiration date)
1 ml 5X Phosphatase Inhibitors (store at -20°C, use by expiration date)
1 ml 2X SDS Sample Buffer (store at 4°C)

Please note this is the buffer validated specifically for our RPPA platform. Please don't use RIPA or any other buffer with NP40 or Triton X detergent. If you wish to make your own buffer for a large number of samples, please reach out to us for further information.

Reagents required, but not supplied by Proteomics Core:

β-mercaptoethanol
1.5 ml microcentrifuge tube with tight cap for lysate aliquot.
We use the microcentrifuge tube from Fisherbrand, catalog number 02-681-320.

Reagent Preparation

RPPA Working Solution (5 ml) Preparation

- Obtain lysis buffer components from the Core and store in an appropriate location. Prepare lysis buffer fresh on the day samples will be lysed.
- Add 1 ml of Protease Inhibitors (5X) and 1ml Phosphatase inhibitor (5x) to the tube containing 3 ml RPPA lysis buffer to create a 5 ml working solution.
- Discard any remaining buffer. Always use fresh RPPA Working Solution.

1X RPPA Sample buffer (1 mL) – make only the volume needed.

475 µL	RPPA Working Solution
500 µL	2x SDS sample buffer.
25 µL	2-mercaptoethanol (2.5% final conc.)

Cell Pellet Direct Lysis Procedure:

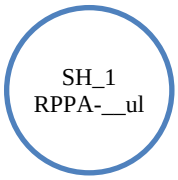
1. Cells should be collected and pelleted by centrifugation. Remove as much residual buffer solution and flash-freeze pellets at -80°C.
2. An accurate cell count is required to determine the total number of cells in the pellet.
3. Thaw cell pellets on ice and add appropriate volume of 1X RPPA Sample Buffer based on the following chart, and then break down the pellet by pipetting.

Cell counts*	Amount of 1X RPPA Sample Buffer
500,000	60 µl
250,000	40 µl
100,000	30 µl
50,000	25 µl

*The cell count here is based on the results from a few breast cancer cell lines tested. For your specific cell line, please use this as a guideline to test and determine the appropriate volume.

4. Incubate pellet with 1X RPPA Sample Buffer at room temperature for 30 min and mix every 10 min.

5. Heat samples for 5 min at 95°C.
6. Spin at 14,000 x g for 15 min at room temperature then transfer supernatant to a new tube. Repeat centrifugation at 14,000 x g for 15 min at room temperature then transfer supernatant to a new tube. Record your total volume. If the supernatant is sticky or hard to collect after the above steps, add an extra 5-10 µl 1X RPPA Sample Buffer before spinning again.
7. If supernatant is still cloudy, please repeat the above spin and transfer step until the supernatant is clear.
8. Please consult the core director for specific project needs if the volume is too low. Otherwise, transfer supernatant into **one** tube and indicate volume in the tube and in the sample submission form (please use 1.5 ml microcentrifuge tube with tight cap to avoid sample loss during heating).
9. Clearly label sample tubes and put them into a freezer/cardboard box labeled with your name/PI name, contact number/date etc.
   Label top of the **tube** as follows:
   PI initials_Auto# (ex: SH_1)



   Label **freezer/cardboard box** as follows:

   Investigator Name/PI name
   Contact number
   Date

10. Store at -80°C until submission.
11. Send your *RPPA_Sample Submission Form* by email **before** you deliver your samples to the Core.

*__DO NOT__ use homemade or any other concentration of SDS Sample Buffer as it may generate higher background based on previous observations.

Please contact us if you have any questions.