

Protocol for Lysis of Cells in Culture for Reverse Phase Protein Array

Reagents provided by Core

3 ml RPPA Lysis Buffer (T-PER based buffer) (store at 4°C)

1 ml 5X Protease Inhibitors (store at -20°C)

1 ml 5X Phosphatase Inhibitors (store at -20°C)

Choose one of the following SDS-sample buffers based on your estimated protein concentration:

1 ml 2X SDS Sample Buffer (store at 4°C)

0.5 ml 4X 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 Core

β -mercaptoethanol

1.5 ml microcentrifuge tube with tight cap for aliquoted lysates.

We recommend using the microcentrifuge tube from Fisherbrand, catalog number 02-681-320.

RPPA Working Solution (5ml) 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 the 1 ml of Protease Inhibitors (5X) and 1 ml Phosphatase inhibitor (5X) to the tube containing 3 ml RPPA lysis buffer to create a 5 ml RPPA working solution.
- Discard any remaining buffer. Always use fresh RPPA Working Solution.

Lysis Procedure (for attachment cells):

1. Remove media from viable cell cultures.
2. Wash cells 3 times with cold PBS. Be sure to remove any remaining PBS as much as possible.
3. Add appropriate volume* of RPPA Working Solution.

Cell counts*	Amount of RPPA working solution
5x10 ⁶	300 μ l
2x10 ⁶	100 μ l

*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 final volume.

4. Scrape cells and transfer cell suspension to a 1.5 ml tube, vortex for 15 sec, and incubate on ice for 30 min, vortexing every 10 min. Alternatively, tubes can be placed on end-over-end rotator for 30 min at 4°C.
5. Centrifuge at 14,000 x g for 15 min at 4°C, then transfer supernatant to a new tube. Repeat centrifugation at 14,000 x g for 15 min at 4°C, then transfer supernatant to a new tube. If the supernatant is still cloudy, repeat spin step and transfer to a new tube until clear.
6. Perform BCA or Bradford assay to determine protein concentration. **Use RPPA Working Solution as the blank and diluent for standards when measuring protein concentration.**
The desired protein concentration is between 1.1 mg/ml – 3 mg/ml.

NOTE: This is a two-step procedure. Cell lysis in RPPA Working Solution is followed by addition of SDS-sample buffer and denaturation of proteins. The entire procedure should be done continuously. Do not freeze lysates in RPPA lysis buffer. The protein concentration assays must be done prior to addition of SDS-sample buffer since SDS interferes with assays. To minimize proteolytic degradation, these steps should be done as quickly as possible.

7. Prepare the lysates for RPPA analysis by adding SDS Sample Buffer, β -mercaptoethanol, and RPPA Working Solution to obtain a final concentration of **0.5 mg/ml**. Depending on your protein concentration, choose one of the tables below to prepare the final RPPA ready samples.

NOTE: Prepare all your samples the same way using the same type of SDS Sample buffer.

For lysates ≥ 1.1 mg/ml	
__ μ l	Lysate
__ μ l	RPPA Working Solution
30 μl	2X SDS Sample Buffer
1.5 μl	β -mercaptoethanol (2.5% final concentration)
60 μl	Total Volume

For lysates < 1.1 mg/ml	
__ μ l	Lysate
__ μ l	RPPA Working Solution
15 μl	4X SDS Sample Buffer
1.5 μl	β -mercaptoethanol
60 μl	Total Volume

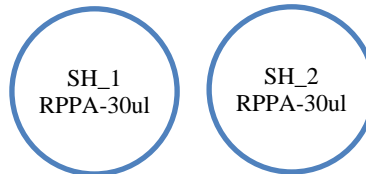
8. Heat samples for 5 min at 95°C.
 9. Bring to room temperature, centrifuge 2 min at 14,000 x g, and collect supernatant.
 10. Aliquot into two tubes (30 μ l for each tube). **Please use a 1.5 ml microcentrifuge tube with tight cap to avoid sample loss during heating.**

If your sample volume is low, please contact us for advice and DONT just distribute it evenly between #1 & #2 tubes!

11. Clearly label sample tubes and put them into a freezer/cardboard box labeled with your name/PI name, contact number/date etc.

Label tubes as follows:

PI initials_Auto# (ex: SH_1)



Label freezer/cardboard box as follows:

Investigator Name/PI name
Contact number
Date

12. Store tubes at -80°C.
 13. Send your *RPPA_Sample Submission Form* by email **before** you deliver your samples to the Core.
 14. Deliver samples on dry ice to the Core.

*Alternatively, cells can be harvested by trypsinization or other methods and collected as a cell pellet prior to lysis. In this case, wash cells twice with PBS by centrifugation and remove all PBS from the pellet. Add an appropriate amount of Working Solution directly to the cell pellet. Suspend the cells, transfer to a 1.5 ml tube, vortex, and proceed as above from steps 3-14.

****DO NOT** use homemade or any other SDS Sample Buffer as it may generate higher background based on previous observations.

*****Use the same SDS Sample Buffer (either 2X or 4X) for all your samples. DO NOT** prepare some samples in 4X and some samples in 2X Sample Buffer.

Please contact us if you have any questions.