

Yael Rosenberg-Hasson
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Supernatants and Homogenates:

Normalization is based on plating equal number of cells.

If experiment exceeds 24 hours consider counting live cells at the end of the experiment for normalization.

Plan to perform a low spin (350 RCF) to pellet intact cells and then collect supernatant avoiding pellet.

We recommend to sub aliquot the supernatant to keep frozen portions for future assays.

Please plan to submit an aliquot of the Media for background level.

Consider running singlets and submit independent biological replicates of your design and conditions.

Samples can be submitted in tubes/vials (low binding) or in 96 well polypropylene plates (low protein binding) is recommended.

Please attach an excel list matching your plates or vials and ensure label is clear and readable.

Ship to address is below:

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(650) 723-4984

Homogenization buffers:

1. Homogenization buffer (Andreasson Lab-):

Final concentration

1% Triton-X 100 500ul

0.5% NP-40 (tergitol) 250ul

25mM Tris HCL Ph7.5 1.25ml of 1M stock

100mM NaCL 1ml of 5M stock

Protease Inhibitors 1:100 500ul from sigma (or tablet from Roche)

PMSF 100X 500ul

Water 46ml

total 50mls

Extraction:

Then add 10 times of tissue weight of lysis buffer, wait few minutes on ice

Sonicate at level 3 for 10 seconds, centrifuge at 14000g, 10mins at 4c

Transfer supernatant (containing protein) to new labeled tubes.

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Measure protein concentration using BCA Assay and Adjust protein concentration to the lowest sample concentration (As I know the lowest protein concentration, they can detect is 0.4ug/ul, Take 120ul for running Luminex. We recommend duplicates for homogenates.

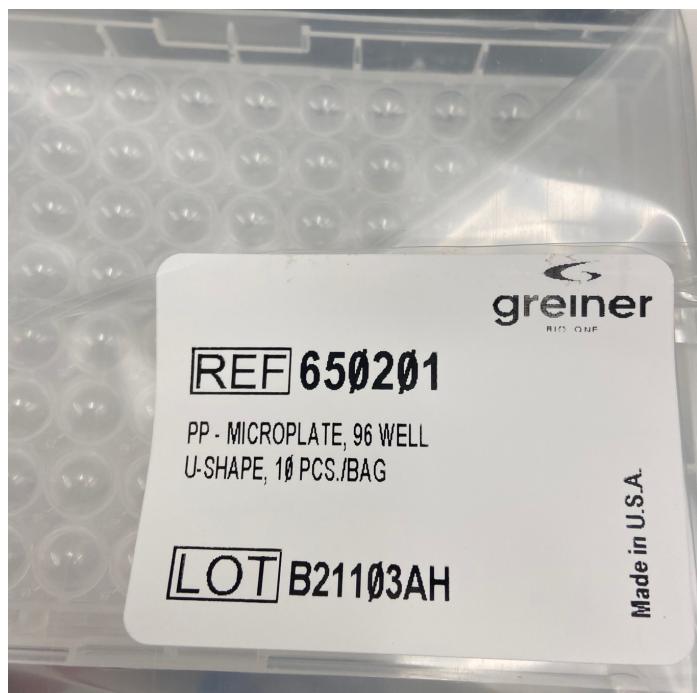
2. Pamela's (Palmer Lab) buffer:

20 mM Tris HCl (ph7.5)
0.5% Tween 20
150 mM NaCl
Sigma protease inhibitors 1:100

3. RIPA Buffer: different compositions by many vendors (least preferred).

Homogenization can be done mechanically and/or using beads for sonication etc.
Make sure to homogenize well and spin the samples at 10000g for 10 minutes.
Plan to measure protein concentration and normalize to equal amount of protein between samples before you freeze aliquots to submit for assays. 2-5ug/ul is a good starting point, 1ug/ul could be sufficient depending on treatment, response and biomarker and tissue. Please ensure the aliquot submitted contain 150 ul of normalized sample.

We suggest testing a few samples as a pilot before you run an entire set of samples collected. That said we recommend running all samples and time points at the same time. Our assay includes 2 replicates per sample, and we can run up to 46 per plate.



Use Low binding polypropylene plates Cat# 650201 or 1.5ml/ 0.5ml vials

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