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Mouse tissue lysate preparation

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We use this protocol and it's working

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Abstract

This protocol is for the preparation of lysate of mouse tissues using a detergent in non-denaturing conditions

Materials

- Ice
- Mouse tissue
- 10-15mL dounce homogenizer
- MilliQ H_20
- 15mL conical tubes
- 1.5mL microcentrifuge tubes
- TRIS
- SDS
- NaCl
- Protease Inhibitor Cocktail
- Heat Block

Mouse tissue lysate preparation

1 Weigh tissue, keep on ice, and prepare 1mL of Homogenization Buffer for approximately every 100mg of tissue.

Homogenization Buffer:
- 25mM TRIS pH 7.5
- 150mM NaCl
- 1x Protease Inhibitor Cocktail

2 Using a tissue homogenizer, submerge your tissue in the appropriate amount of homogenization buffer (1mL/100mg) in the homogenizing vessel. Homogenize the tissue thoroughly. Be sure to keep the tissue grinder submerged while homogenizing to avoid frothing.

3 Transfer the homogenate to a 15mL conical tube and add 0.5ul of benzonase per mL of homogenate.

4 Using a concentrated stock solution of SDS, bring the homogenate up to 2% SDS. Use the equation below to determine the volume of SDS to add from your stock solution to your homogenate in order to bring it to 2%. The volume of stock solution to add is as follows:

$$\mathbf{V_{stock} = V_{start} / ([stock]/[target] - 1)}$$

where V_{stock} is the volume of stock solution to add, V_{start} is the total amount of homogenate you start with, [stock] is the concentration of your stock solution, and [target] is your final desired or target concentration (in this case 2%)

5 Take a small sample for BCA analysis (plan to dilute ~5x for BCA).

6 Add concentrated loading sample buffer and bring to 1x. Aliquot the lysate into microcentrifuge tubes. Boil tubes @ 95°C for 3 minutes.

7 Spin samples briefly at max speed for 10 seconds to spin down condensation left on the caps. Load samples immediately in a gel or store @ -20°C.