



شرکت سلول بافت زیست (سبز)

Protein Extraction Protocol

1. Freeze tissue in liquid nitrogen after excision.
2. Add some liquid nitrogen to the tissue in a mortar and powder it with a pestle.
3. Move the powdered tissue to a tube and add RIPA buffer to it while it is still cold.
4. Add PMSF (20 μ l of 200mM PMSF for 1 ml of RIPA; Final concentration: 4mM)
5. Vortex for 60 second.
6. Put on ice for 45 minutes and shake it every few minutes.
7. Centrifuge at 14000 g for 10 min at 4 °C and move the supernatant to a new tube.
8. Store the samples in -80 °C until needed.

RIPA buffer:

- Tris-HCl 50 mM, pH: 7.4
- NaCl 150 mM
- EDTA 1mM
- Triton X-100 1%
- SDS 0.1%

Note:

- Use at least 5 ml of RIPA per gram of tissue.
- The half life of PMSF in aqua's solutions is only about 30 minutes. So prepare **fresh** 200mM PMSF in isopropanol and store it at -20 °C until use.