

Protein Extraction from Mice Xenograft Tumor

Huan Pang

Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, USA

*For correspondence: pang_huan@hotmail.com

[Abstract] Proteins from frozen tissue such as xenograft tumor need to be extracted efficiently without being degraded to make the best use of a limited resource and to ensure that an accurate representation of proteins in selected tumor tissue can be obtained. This protocol describes how to extract proteins from xenograft tumors in mice.

Materials and Reagents

1. NaCl
2. Tris
3. EDTA
4. NP-40
5. EGTA
6. NaF
7. Beta-glycerophosphate
8. Sodium pyrophosphate
9. Sodium vanadate
10. Isoflurane
11. PMSF
12. Laemmli buffer
13. Liquid nitrogen
14. Phosphatase inhibitor 1/2 (Sigma-Aldrich, catalog number: P2850, P5726)
15. BCA kit (Thermo Fisher Scientific, catalog number: 23227)
16. Lysis buffer(see Recipes)

Equipment

1. Centrifuges
2. Sonicator
3. Western blotting equipment

Procedure

1. Anesthetize the mice using isoflurane.
2. Rapidly remove the tumor from live mice.
3. Flash freeze the xenograft tumor in liquid nitrogen, then store at -80 °C or proceed to direct preparation of the samples.
4. Break frozen tissue (present in liquid nitrogen) into several small pieces, and weigh one or two pieces.
5. In dry ice, smash tissue in to smaller pieces, then add lysis buffer 2.5 µl/mg. Homogenize on ice.
6. Sonicate for 5 sec at low setting on ice. Incubate on ice for 30 min.
7. Spin at 13,000 rpm for 15 min at 4 °C.
8. Transfer supernatant to a new tube.
9. Dilute samples and perform protein assay using Pierce's BCA kit.
10. Adjust the concentration to an identical level.
11. Add 5x Laemmli buffer and boil for western blot.

Recipes

1. Lysis buffer
 - 150 mM NaCl
 - 10 mM Tris
 - 1 mM EDTA
 - 1% NP-40
 - 1 mM EGTA
 - 50 mM NaF
 - 40 mM beta-glycerophosphate
 - 10 mM sodium pyrophosphate
 - 1 mM sodium vanadate
 - 8 mM PMSF
 - phosphatase inhibitor 1/2

References

1. Dignam, J. D. (1990). [Preparation of extracts from higher eukaryotes.](#) *Methods Enzymol* 182: 194-203.

2. Pang, H., Flinn, R., Patsialou, A., Wyckoff, J., Roussos, E. T., Wu, H., Pozzuto, M., Goswami, S., Condeelis, J. S., Bresnick, A. R., Segall, J. E. and Backer, J. M. (2009). [Differential enhancement of breast cancer cell motility and metastasis by helical and kinase domain mutations of class IA phosphoinositide 3-kinase](#). *Cancer Res* 69(23): 8868-8876.