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Seifert Lab SOP – Protein Extraction

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We use this protocol and it's working

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Abstract

This protocol outlines the basics of protein extraction from ear pinna tissue.

Troubleshooting

Tissue Collection

1 Anesthetize the animals, harvest tissue and dissect the 4mm punch.

2 Hold the tissue in the forceps and rinse it quickly in HBSS/PBS.

3 Transfer it into the freshly labelled 1.5ml Eppendorf tube and snap freeze in liquid nitrogen.

4 Transport to lab and store it at -80°C freeze for further use.

Protein Extraction

5 Note: Before starting, set the centrifuge at 4°C.

6 The collected tissue is taken out of -80°C freeze and kept in ice bucket.

7 Add 200-400ul of cold protein extraction buffer per sample.

7.1 Cold Extraction buffer: In 1ml of RIPA buffer, freshly add 10ul each of protease inhibitor cocktail, Na-orthovanadate and PMSF.

8 Use the manual homogenizer to dislodge the tissue (10-15 times). Put it back in ice.

8.1 Homogenize the dislodged tissue using sonicator.

8.2 You require 1, 15ml falcon of DI water and 1, 15ml falcon of 70% ethanol.

8.3 Conditions on sonicator machine: Pulse-Continues, Control cycle-4 and duty cycle-40%.

8.4 First wash the sonicator twice with 70% ethanol (10 sec each) followed by twice with DI water (10 sec each).

8.5 Now sonicate your protein sample-3 times with 20-25 sec pulse followed by 30 sec in ice.

8.6 In between the samples, wash the sonicator with water followed by 70% ethanol for 5 sec each.

8.7 After finishing extracting protein from all the samples, clean the sonicator by twice with DI water (10 sec each) followed by twice with 70% ethanol (10 sec each).

9 Centrifuge the samples at 13,000 rpm for 15 min at 4°C.

10 Collect the supernatant in freshly labelled 1.5ml Eppendorf tubes. From here, either store it at -80°C freeze for further use or go for protein quantification.

Protein Quantification

11 We will take 96-well plate and add different concentrations of BSA standards (2.5ug, 5ug, 10ug, 20ug and 40ug).

12 The protein samples will be loaded as 2ul or 5ul/well in triplicates.

13 Now add 100ul of BSA reagent in each well.

14 Go for plate reading according to their saved protein estimation protocol.

15 The estimated protein will be calculated and used accordingly for western blot.