Western Blot Analysis: Preparation of Protein Extracts

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Reagents

Aprotinin
Sigma, Cat. A1153
EDTA 0.5 M
Quality Biological, Inc., Cat. 351-027-100
HEPES, pH 8
Isopropanol (2-Propanol)
Leupeptin
Sigma, Cat. L2884
NP-40
Sigma, Cat. NP-40
1X Phosphate Buffered Saline, pH 7.4
Invitrogen Corp., Cat. 10010-023
PMSF
Sigma, Cat. P7626
Sodium chloride (NaCl)
Mallinckrodt, Cat. 7581
Sodium fluoride (NaF)
Sigma, Cat. S1504
Sodium orthovanadate (Na_3VO_4)
Sigma, Cat. S6508
Sodium pyrophosphate ($Na_4P_2O_7$)
Sigma, Cat. S6422
Tris, pH 7.5 1 M
Quality Biological, Inc., Cat. 351-006-100
Trypsin
Tubes, Beckman Polyallomer microfuge
Beckman, Cat. 357448

Preparation

Aprotinin [1000X]
1 mg/ml in 0.01M HEPES pH 8
Store at -20˚

Leupeptin [1000X]
1 mg/ml in sterile water
Store at -20˚C

PMSF [10X]
1.74 mg/ml in isopropanol
Store at –20˚C

TNE Lysis Buffer

1 M Tris, pH 7.5	2.5 ml	f.c. [50 mM]
0.5 M EDTA	0.2 ml	f.c. [2 mM]
$Na_4P_2O_7$	111.5 mg	f.c. [5 mM]
Na_3VO_4	0.9 mg	f.c. [100 μM]
NaF	10.5 mg	f.c. [5 mM]
NaCl	438.3 mg	f.c. [150 mM]
100% NP-40	0.5 ml	f.c. [1%]

*Bring volume to 50 ml with sterile water
Store at 4˚C

TNE Lysis Buffer + Protease Inhibitors (PIH)
Dilute fresh from Stock into desired aliquot of TNE Lysis Buffer:

Aprotinin	1:1000
Leupeptin	1:1000
PMSF	1:10

Procedure

1. Wash cells 1 x with 1X PBS.

2. Trysinize cells to remove from flask/plate.

3. Add 10 ml media containing FBS to inactivate trypsin & pellet cells at 1200 rpm.

4. Resuspend cells in 1 ml 1X PBS and transfer to Beckman Polyallomer microfuge tubes.

5. Pellet cells at 1000 rpm in microfuge.

6. After aspirating PBS keep cells on ice from this point on.

7. Resuspend pellet in 100 μl TNE Lysis Buffer + freshly added protease inhibitors (PIH).

8. Lyse cells on ice 30 min.
   (Samples can be stored at -80˚C at this point until further processing)

9. Centrifuge samples at 45,000 x g at 4˚C for 45 min in ultracentrifuge.

10. Transfer supernatant containing soluble proteins to new tubes.

11. Determine protein concentration (See "Cell Lysate Quantitation" protocol)