Preparation of Protein Extracts for 2D Gel-analysis

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Reagents

Aprotinin
Sigma, Cat. A1153
Benzamidine
Sigma, Cat. B6508
Hepes, pH 8
Isopropanol (2-Propanol)
Leupeptin
Sigma, Cat. L2884
Phenylmethylsulfonyl fluoride (PMSF)
Sigma, Cat. P7626
1X Phosphate Buffered Saline (PBS), pH 7.4
Invitrogen Corp., Cat. 10010-023
Sodium fluoride (**NaF)**
Sigma, Cat. S1504
Sodium orthovanadate (**Na_3VO_4)**
Sigma, Cat. S6508
Sodium pyrophosphate (**$Na_4P_2O_7$)**
Sigma, Cat. S6422
Water, sterile

Preparation

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

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

PMSF [50X]
1.74 mg/ml in isopropanol
Store at -20˚C

2-D Lysis Buffer

1X PBS	100	ml	
$Na_4P_2O_7$	223	mg	f.c. [5mM]
Na_3VO_4	1.8	mg	f.c. [100μM]
NaF	21	mg	f.c. [5mM]
Benzamidine	13	mg	f.c. [830μM]

Store at 4°C

2-D Lysis Buffer + Protease Inhibitors (PIH)

Dilute fresh from Stock into desired aliquot of 2-D Lysis Buffer:

Aprotinin	1:1000
Leupeptin	1:1000
PMSF	1:50

Procedure

1. Wash cells 2 x with 1X PBS pre-chilled to 4˚C.

2. Scrape cells off plate into ice cold 2-D Lysis Buffer + PIH. Volume of 2-D Lysis Buffer + PIH should be determined by the size of plate/flask in which the cells are growing; 2.5 ml is sufficient for T-150 flask.

3. Pellet at 660 x g for 3 min (about 2000 rpm in clinical centrifuge) at 4˚C.

4. Resuspend pellet in 1 ml 2-D Lysis Buffer + PIH, transfer to pre-weighed eppendorf tube and pellet at 2700 x g for 5 min (about 5700 rpm in microfuge) at 4˚C.

5. Aspirate off 2-D Lysis Buffer and determine weight of pellet.

6. Store at -80˚C until further processing for 2-D gels.

Notes

1. If wet weight (WW) of pellet is 5-10 mg (~10 million cells) it is usually possible to obtain one acceptable 2-D gel. It is always recommended to obtain cell pellets of more than 15 mg, so it may be necessary to harvest and pool together many plates in order to obtain a sufficient pellet.