

ULTIMATE FREEZE-THAW LYSIS FOR MAMMALIAN CELLS

This extraction procedure was developed by the Simm Laboratory and described in Rudolph *et al.*, 1999 (*Anal Biochem* **269,** 66-71). It's a great way to make very concentrated extracts for Western blotting, and is particularly useful when studying low-abundance proteins (like Myc!). The protocol is described for harvesting mammalian cells from 6 or 10 cm tissue-culture dishes.

A. CELL HARVEST.

1. Remove media from cells. Wash in ice-cold PBS.

2. Scrape cells in 1 ml of ice-cold PBS. Transfer to 1.5 ml microfuge tube.

3. Pellet cells at top speed in microfuge (45s in cold-room).

4. Resuspend cell pellet in FT LYSIS Buffer (25-40 µl per confluent 6 cm dish, 50-80 µl per confluent 10 cm dish). Resuspend by pipeting up and down.

5. Drop tube in liquid nitrogen to freeze. Thaw sample on ice (thawing takes a few minutes). Vortex briefly.

6. Repeat freeze-thaw cycle two more times.

7. Thaw cell lysate. Add 250 U of Benzonase (Merck) to digest DNA. Mix. Incubate at room temperature for 10 minutes.

8. Measure protein concentration.

9. Add an equal volume of 2xLaemmli Buffer prior to analysis by SDS-PAGE. Heat at 95oC for 10 minutes. Load immediately on gel. (Note that these samples must be loaded while still warm, because the salt in the FT Buffer will cause the SDS in the Laemmli to precipitate when cold).

B. SOLUTIONS.

FT LYSIS BUFFER	**For 100 ml**
600 mM KCl	20 ml 3 M KCl
20 mM Tris-Cl (pH 7.8)	2 ml 1 M Tris-Cl (pH 7.8)
20% Glycerol	20 ml glycerol
Add fresh each time:	
0.4 mg/ml Pefabloc	1:100 of 40 mg/ml stock
10 μg/ml Leupeptin	1:1000 of 10 mg/ml stock
10 μg/ml Pepstatin	1:500 of 2 mg/ml stock
5 μg/ml Aprotinin	1:2000 of 10 mg/ml stock