Objective.

Protocol for cell lysis, designed for whole proteome characterization by LC-MS/MS. The protocol uses nonionic detergent (NP-40) and is not suitable if your goal is to specifically analyze membrane proteins.

Please follow this protocol and provide the Proteomics Resource Center with 10-50ug of quantified protein.

The protocol is provided by Maria Passarelli.

Lysis buffer	**Concentration**	
Tris-HCl	20mM	
KCl	100mM	
NP-40	0.5%	Do not substitute with SDS
cOmplete Protease Inhibitor (EDTA free)	1 pellet per 10mL	

Other equipment
Centrifuge
Sonicator
BCA assay
Equipment for running SDS-PAGE

Procedure – Cell lysis

		Examples, Tricks & Comments
1	Prepare lysis buffer as described above	
2	Resuspend cell pellet in appropriate volume of lysis buffer (400 uL minimum for sonication) in 1.5 mL Eppendorf tube	
3	Vortex for 5 seconds	
4	Sonicate samples in ice (amplitude: 40%) at 5 second pulses with 30 second breaks, 4 pulses total	
5	Centrifuge samples at maximum speed for 10 minutes at 4C.	
6	Transfer supernatant to new tubes.	

Procedure – Quality Control

1	Withdraw an aliquot from all samples and measure concentration using BCA or comparable assay.	
2	Withdraw 5 ug from each sample and run SDS-PAGE. Stain with Colloidal blue. If all lanes look similar, your sample is ready to submit.	*If lanes do not look similar, it may be caused by contamination of a single high-abundant protein. Repeat lysis.*