Glycerol Gradient General Protocol

Cell Culture and Drug Treatment:
Culture cells in three T-150 flask at 37 C in 24 mL media
Drug treat the cells with the desired concentrations and time courses
Scrape harvest the cells and transfer the media and cells from both flasks to a 50 mL conical tube (on ice)
Centrifuge cells at 2K for 5 minutes in the J6 centrifuge at 4 C
Wash cells with 4 mL 1x ice cold PBS
Repellet the cells at 4K for 5 minutes in the J6 centrifuge at 4 C
Resuspend cells in 200 uL 150mM NaCl lysis (Add PMSF, DTT, and the protease inhibitor) buffer, gently vortex, transfer to microfuge tubes, and incubate on ice for 10 minutes with intermittent vortexing
After lysis, pellet cells at full speed in a microcentrifuge for 5 min at 4 C
Remove and save the supernatant

Lysis Buffer:

	Stock	Final	50mL	Add to 1mL of Stock Lysis buffer
HEPES	0.5M	10mM	1	
MgCl2	1M	2mM	0.1	
KCl	1M	10mM	0.5	
NP-40	100%	0.50%	0.25	
EDTA	0.4M	0.5mM	0.0625	
NaCl	5M	150mM	1.5	
H2O			46.5875	
DTT	1M	1mM		1 uL
PMSF	100%	0.10%		1 uL
Protease Cocktail	25u/mL	1u/mL		40 uL

Glycerol Gradient Solutions

	Stock []	Final []	45%	5%
HEPEs	0.5 M	10 mM	20	20
MgCl2	1 M	2 mM	2	2
KCl	1 M	10 mM	10	10
Glycerol	100%		450	50
EDTA	0.4 M	0.5 mM	1.25	1.25
NaCl	5 M	150 mM	30	30
H2O			486.75	886.75

Filter solutions at .22 um

Glycerol Gradient
Making the gradient

Create the gradient using an FPLC program, a gradient apparatus, or by making ~10 different glycerol solutions and layering them in a 5mL ultracentrifuge tube

Centrifuge
Use the SW55ti rotor
Clean out the rotor vials with a kim wipe
Turn on the centrifuge
Set the desired speed (45,000 rpm ~200,000xg)

Loading samples
Load the samples on top of the gradients slowly and evenly
Place the pipet tip on the side of the gradient tube and slowly layer 200uL of sample ontop of the gradient
After applying sample, carefully load the gradient into the centrifuge vial
Make sure that the rotor is balanced correctly – Its really bad if its not balanced

Running gradient
Gradients are run for 16-18 hours overnight
Turn the vacuum on
Set brake to 800
Turn hold on
Set temp to 4C
Press start once the samples and rotor are loaded into the centrifuge