

Protein Preparation from Transfected Cells

Transfection for protein lysates: 10 cm plates, 6 μg DNA per plate, plasmid stocks 0.1 $\mu\text{g}/\mu\text{l}$

Example set-up:

Empty vector	40 μl	Plasmid	20 μl
Vector-GFP	20 μl	Empty vector	20 μl
		GFP	20 μl

1. Mix DNA
2. Mix serum free media (SFM) + FuGene6 (Roche), incubate 10 min @ RT
Per 10 cm plate:
 - 15 μl FuGene
 - 485 μl SFM
 - 500 μl total for each plate
3. Mix DNA + SFM/FuGene, incubate 10 min @ RT
4. Pipet onto cells

Harvesting Cells

Following transfection @ appropriate timepoint (i.e., 48-72 hrs):

1. Remove media, wash with cold PBS
2. Put cells on ice, add 1.5 ml PBS
3. Scrape cells off plate, spin in 1.5 ml tube 5 min @ 10,000 rpm @ 4°C
4. Wash pellet 2x with PBS
5. Resuspend in 0.5 ml RIPA + complete mini protease inhibitor tablet (Roche)
 - 10 ml RIPA + 1 tablet, store @ 4°C for two weeks
6. Freeze @ -80°C
7. Thaw on ice
8. Pass through 22 g needle
9. Spin @ 14,000 rpm, 4°C for 20 min
10. Keep supernatant (pellet is debris); store @ -20°C after measuring concentration via Bradford Assay

Bradford Assay

Dilute Bradford reagent 1:5 in dH₂O

Place 1 ml of reagent in cuvette per sample

Add 1 μ l sample to 1 ml reagent, pipette up and down

Remember blank!

Incubate @ RT ~ 30 min

Spec in Fero lab or Murdoch room