

³⁵S-Methionine Metabolic Labeling

1. Seed cells (250,000 cells/well for 293T) in 6-well plate. Put each time point on different plate.
2. Transfect cells. Wait 18-24 hrs.
3. **Wash cells 2X in labeling media (Without Met and Cys, but add L-Glut).**
4. **Add 1 mL media to each well. Incubate 1 hour.**
5. **Add 5 μ L (50 μ Ci/mL) of ³⁵S -met. Incubate 4 hours.**
6. **Wash cells with chase media 2X.**
7. **Incubate in 2 mL chase media for chase time.**
8. **Wash cells in PBS 2X.**
9. **Lyse cells in RIPA + Complete.**
10. Immunoprecipitate with anti-GAL4 antibody.
11. Count activity of lysate— 1 μ L sample + 1 mL liquid scintillation cocktail.
12. Run gel (about 10,000 counts per lane).
13. Fix gel in 40% methanol, 10% acetic acid for 15 minutes.
14. Coomassie stain, if desired.
15. Use Entensify (NEN) part A 45 min.
16. Use Entensify part B 45 min.
17. Dry gel for 1-2 hours.
18. Autoradiograph—use ³⁵S intensifying screen and X-OMAT film. -70C.