

Metabolic Labeling of Cells with ³⁵S

- 1) Transfer to a 24 wells plate the desired colonies.
- 2) Once the cells are attached (at least 8 hours after trypsinizing them) add ~1 ml of DME (met⁻, cys⁻).
- 3) Add 3 μl of Met ³⁵S, Cys ³⁵S trans label.
- 4) Incubate at least 5 hours at 37°C (Put plate(s) inside a tupperware, containing a beaker with activated charcoal, and close loosely).
- 5) Collect the media into 1.7 mL tubes and add 5 μl of the desired antibody.
- 6) Incubate at least 1 hour using the mixer located at cold room.
- 7) Centrifuge briefly.
- 8) Add 100 μl of a 75:25 protein A:50mM Tris mixture.
- 9) Incubate at least 1 hour using the mixer located at cold room.
- 10) Centrifuge briefly.
- 11) Remove the supernatant using a 5 ml syringe with a 26 1/2 G needle.
- 12) Add 1 mL of 1 x PBS/0.05% Tween to the beads.
- 13) Mix by tapping and by inverting it a couple of times.
- 14) Centrifuge for 30 seconds at 6,000 RPM.
- 15) Repeat steps 12-14 two more times.
- 16) Repeat steps 12-14 one more time but instead of adding 1 x PBS/0.05% Tween add 1 x PBS.
- 17) Take as much volume as you can out of the beads and add 10 μl of 50 mM tris and 15 μl of non-denaturing loading buffer.
- 18) Boil samples at least 5 minutes.
- 19) Load as much as you can on an SDS-PAGE (I use 15% Gels) gel.
- 20) After running the gel incubate it 45 minutes on 30% ETOH:10% HOAc.
- 21) Dry gel for 1.5-2.0 hours at 70 C.
- 22) Expose O/n to a phosphoimager screen.