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# ULTIMATE MAMMALIAN CELL PULSE-CHASE

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This assay is performed to directly measure the stability of a protein in mammalian cells. The assay works well with transiently-expressed proteins, providing they have multiple-methionine residues and can be expressed at high levels in transfected cells. For proteins that can only be expressed at low levels (or proteins that have a few methionine residues) we use the methionine-fortified HAM tag (available from Bill Tansey: tansey@cshl.edu) to facilitate labeling. For detection of endogenous proteins, you may need to increase the amount of $^{35}$S-labeled methionine (up to 500 µl per 6 cm dish). Labeled proteins can be recovered by denaturing immunoprecipitation (our favorite—see 'Ultimate Mammalian Denaturing IP' protocol) or non-denaturing immunoprecipitation (see 'Ultimate Mammalian Non-Denaturing IP protocol).

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## A. CELL SET-UP.

This protocol is designed for use with ~50% confluent 6xcm dishes of cells. If cells are to be transfected, they should be transfected 24-40 hr before the labeling begins. Labeling is done in methionine- and cysteine-free DMEM—if your cells cannot grow in DMEM, you may substitute another methionine- and cysteine-free media. Typically, each pulse-chase requires four plates of cells—one for the zero time point (pulse) and three for the subsequent time points. This protocol is described for four different pulse-chase determinations (ie., total of 16 plates).

## B. PULSE-CHASE.

Prepare:
(i) 500 ml warm PBS.
(ii) 20 ml warm Met/Cys-free DMEM/5% dialyzed FCS (with Gln if needed). This DMEM and dialysed FCS come from Gibco.
(iii) 900 µl of warm Tran$^{35}$S-label (NEN).
(iv) 100 ml of regular DMEM + FCS + 2 mM Methionine + 2 mM Cysteine.
(v) 100 ml ice-cold PBS.
(vi) "Shoebox" for labeling.
(vii) 16 labeled microfuge tubes (on ice).
(viii) Disposable cell scrapers.
(ix) Liquid nitrogen.
(x) Stopwatch.
(xi) Disposable $^{35}$S-waste (liquid and sharps).

1. Aspirate media from each plate of cells. Wash cells 2 times in 5 ml warm PBS.

2. Aspirate PBS. Add 1 ml warm DMEM (met/cys-free)/5% dialyzed FCS. Incubate cells for 30 minutes at 37ºC. This is the starvation period.

3. Add 50 µl of Tran$^{35}$S-label (at roughly 10 mCi/ml; equals 500 µCi total) to each plate. Place cells in shoebox and incubate for 40 minutes at 37ºC.

4. Carefully remove radioactive media. Wash cells on plate with 2 ml warm PBS. Remove PBS. Add 5 ml warm DMEM+FCS+2 mM Methionine+2 mM Cysteine to all plates except the zero "0" samples (5 plates total). Start timer.

5. Immediately remove all "0" plates. Return other plates to 37ºC.

6. Remove media from "0" plates. Wash cells with 5 ml ice-cold PBS. Remove PBS. Scrape cells in 0.5 ml ice-cold PBS. Transfer to labeled microfuge tube and spin 45 seconds in the cold.

7. Carefully remove supernatant. Freeze cell pellet in liquid nitrogen and store at -80ºC until ready to perform the immunoprecipitation.

8. Perform similar collections at different points during the chase (30, 60, 90 minutes is a good starting point).

Use the ULTIMATE MAMMALIAN CELL DENATURING IP or ULTIMATE MAMMALIAN CELL NON-DENATURING IP protocol to recover your labeled protein.

**Note that the Met/Cys-free DMEM needs to have glutamine added. Also, to the chase media, add Met and Cys fresh (from 200 mM stocks in water) just before the chase.**