Metabolic Labeling (a la Mickey)

Special Stuff you need to have long before you start:

Dialyzed FBS: Serum dialyzed exhaustively against hepes-buffered-saline (10mM HEPES pH 7.4/ 150mM NaCl) or PBS, and then sterile filtered, aliquoted, and stored at -20˚C

1500X methionine/cysteine: 0.3M methionine and 0.3M cysteine in ddH2O, sterile filtered and stored at -20˚C

Met-/Cys- medium: DMEM without methionine or cysteine (from Gibco/BRL or other commercial source)

Tran35S-Label or other source of metabolic label

Reagents to prepare with the above shit and other stuff:

Met-/Cys- Complete Medium
DMEM without methionine or cysteine
3-5% dialyzed FBS
glutamine
pen/strep
10-25mM HEPES pH 7.4

Chase medium, warm and ice cold
Normal, complete medium with serum
15X methionine/cysteine (add 1/100th volume of 1500X met/cys)
10mM HEPES

Ice cold PBS

Hood space and a 37˚C water bath

Procedure:

1. If using adherent cells, trypsinize cells from dish or flask in the usual manner;
   - resuspend in ~10ml of complete medium (with FBS), transfer to a centrifuge tube, and spin out cells at 1000-1500 RPM in the table-top centrifuge for 5-7'
   - if using non-adherent cells, simply spin them out: usually 5-10x10^6

2. Remove all traces of medium
    - resuspend cells in complete met-/cys- medium to ~5x10^6 cells/ml (this means about 1 10cm dish in ~2ml total).
    - incubate at 37˚C for 15-30' for transient HeLa or COS cells (high expressors of whatever you are looking for); 30-60' for stables or other cell lines (low expressors of whatever you are looking for).

3. In the meantime, prepare the labeling medium:
    - for pulse/chases, I prefer to use ~2mCi/ml of Tran^{35}S-Label or other ^{35}S-methionine source; for long-term labeling (2 hrs or more), use 0.25 0.5mCi/ml. For a 10cm dish-ful of cells, 0.5-1ml is fine. (For these, you could actually just add the ^{35}S-met straight to the pre-incubating cells). For non-adherent cells, shoot for 5-10x10^6 per ml.
    - keep medium at 37˚C until ready to add

4. At the end of the pre-incubation, spin out cells.
    - remove medium
    - resuspend in labeling medium, and incubate at 37˚C for however long you want (a typical pulse is 30'); shake tube every now and then during the labeling
    - at this point, it may be convenient to aliquot cells into pulse and chase samples
5. At the end of the labeling:
    - cool cells on ice
    - spin cells out at 4˚C
    - resuspend chase samples in warm chase medium (1ml or more is convenient, usually); incubate at 37˚C for however long you want.
        - it may be convenient to separate multiple chase samples either at the beginning, i.e. here, or later on, depending on your preference and/or space in the water bath
    - resuspend pulse sample in ice cold chase medium as a 1st wash

6. At the end of the chase (and the pulse sample);
    - spin out cells at 4˚C
    - remove medium; save if necessary
    - resuspend cells in a reasonable volume of ice cold PBS or Tris buffered saline, and spin out again
    - discard PBS wash and freeze cell pellets on dry ice for storage, or go ahead and lyse cells directly as desired for other procedures