

Labelling cells with ³⁵S methionine

You will need

1. DMEM minus methionine (Gibco-BRL) with glutamine and pen-strep added
2. dialysed FBS (dialyse against PBS for several hours)
3. ³⁵S methionine

For one 100mm dish of cells

A. To label overnight:

1. remove medium, add 5ml DMEM - met, incubate cells for 30min
2. remove and add 5ml DMEM - met + 10% dialysed FBS + 0.25mCi ³⁵Smet incubate 16hours. Be sure to label dishes with radioactive tape!
3. remove medium and prepare an NP40 lysate for immunoprecipitation.

B. To pulse label for 2hrs:

1. remove medium, add 5ml DMEM - met, incubate cells for 30min
2. remove and add 5ml DMEM - met + 0.25 to 1mCi ³⁵Smet, incubate for 2hours
3. remove medium and prepare an NP40 lysate for immunoprecipitation.

Dispose of all radioactive waste appropriately!