^{32}P ORTHOPHOSPHATE AND ^{35}S METHIONINE/CYSTEINE LABELING OF TISSUE CULTURE CELLS

^{32}P Labeling of Tissue Culture Cells

This methodology is widely used to study the phosphorylation of proteins. Cells incubated with radiolabelled phosphate incorporate this into the cellular ATP pool from which it is utilized for various biological reactions including phosphorylation. High activity labeling of proteins is achieved by labeling the cells in media which is free of phosphate for an extended period of time (a few hours to overnight). Longer incubation periods result in the labeling of DNA and RNA; however, these molecules are typically removed from protein during purification by immunoprecipitation.

As in any experiment with tissue culture cells, the cells should be dense and actively growing during this procedure.

1. *For adherent cells*- Remove the regular growth media and wash the cells twice with phosphate deficient media (available from Gibco in many varieties). Replace with phosphate deficient media using the smallest possible volume (for 60 mm plates use 1-1.5 ml and for 100 mm plates use 2.5-3.0 ml). If the labeling period is long consider including serum which has been dialyzed against phosphate deficient media. ^{32}P orthophosphate is then added to approximately 1mCi/ml. Labeling times depend on the abundance of the protein of interest as well as the kinetics of phosphorylation and must be determined by experimentation. Longer periods result in higher background which may or may not be reduced during the subsequent purification steps.

2. *For suspension cells*- Spin down the cells and replace the growth media with phosphate deficient media. Since the volume is larger the specific activity of labeled phosphate in the media will be much lower unless a great quantity of label is added. It is much more feasible to wait another few days for the result on film than to waste money and reagent. *HeLa S3 spinner cells labeled for only a few hours at 50μCi/ml yield HSF1 which is sufficiently labeled for study.*

^{35}S Methionine/Cysteine Labeling of Tissue Culture Cells

Media deficient in met, cys, or both is available from Gibco or ICN. Tran^{35}S-label^{TM} from ICN is good for labeling cysteine and methionine residues. This label is also very inexpensive because the cost arises in purifying the two residues apart from each other. Labeling the residues individually is much more expensive due to the cost of purification. Typically, labeling is performed at a final radiochemical concentration of 0.1-0.5 mCi/ml for 4-5 hours. Obviously, this depends on the abundance and turnover of the protein of interest.

References:

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