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INTERNAL RADIOLABELLING OF MONOCLONAL IMMUNOGLOBULINS

This protocol is designed for obtaining labeled hybridoma Ig chains which can be analyzed by SDS-PAGE and auto- or fluorography. ^{14}C labeled supernatants can be run on SDS-PAGE (20 µl) and analyzed directly by autoradiography. Higher specific activity labeling is used for competition assay (see separate protocol).

1. Transfer 0.1 ml of healthy, cultured cells at near-maximum density (0.5 to 1 x 10^6/ml) to a sterile 1.5 ml conical tube. If the cells have not multiplied to this density at the time of radiolabelling, transfer a larger volume. In general, a rough estimate of concentration based on turbidity can be made and a volume transferred to give a total of about 10^5 cells.

2. Centrifuge the tube at 200 x g for 5 min at 10°C. Aspirate away all supernatant using a pasteur pipette with a constricted tip while taking care not to remove the pelleted cell mass.

3. Wash cells twice in 1.0 ml of leucine-free medium (see 'Preparation of Met-free and Leu-free Medium' in the Procedures Notebook). The Leu-free medium should contain Gln, gentamycin or P/S, and 10% dialyzed FCS.

4. During washing, prepare 100 µl per cell type of labelling medium.

Important Note: Radioactive compounds must never be handled in a forward-flow laminar hood. Therefore, preparation of isotopes, addition of cells, and all subsequent steps must be carried out on a regular bench, maintaining sterile technique as much as possible until the end of the labelling period.

For ten samples, use:

^{14}C-L-leucine, 100 µCi/ µl (or ^{3}H-L-leucine, 1 mCi/ml)	50 µl
10 x Earle's Balanced Salts	5 µl
Leu Free Medium + Gln, 10% dialyzed FCS	1000 µl

(Obviously, other labelled amino acids, together with the appropriate amino acid free medium, may be substituted).

5. After the last wash, resuspend cells in 100 µl of the labelling medium prepared in Step 4 and transfer to 96 well plates. Add distilled water to surrounding wells for humidity. Incubate overnight at 37°C, 10% CO_2.

6. Transfer to microfuge tubes and centrifuge. Remove and store the supernatant at -35°C. Discard the cell pellet. (see also 'High Specific Activity ^{3}H-labelled Ig for Cross Inhibition Experiments').