Metabolic Labeling Using 32P

Protocol for metabolic labeling with orthophosphate. Developed by Trinna Cuellar. Last updated 2008 October 22.
Materials:

Cells and hot stuff
Methods:
Protocol:

1. Prepare the work area for use with 32P by placing all necessary items (e.g., pipets, Geiger counter) within easy reach. Place the flask with the cells behind a beta shield.
CAUTION: The initial 32P labeling of cells may involve large amounts of 32P (see safety precautions in UNIT 10.9 and in critical parameters and troubleshooting). Rigorous precautions should be taken to avoid exposure of individuals and contamination of laboratory equipment. Be sure to think ahead to carry out the following steps in an efficient but unrushed manner.

2. Place the source vial containing 10 mCi of 32P in a vial beta shield. Open source vial using forceps to remove protective cover. Resuspend 32P in 0.5 ml phosphate-free RPMI using a transfer pipet. Add 32P to the cells, mix, and incubate 90 min.
CAUTION: To protect yourself and others from the 10 mCi of 32P during transport, place the container with the cells over absorbent paper inside a Ã¢â€¦Å“-in.-thick Plexiglass box. Place the Plexiglass box behind a lucite screen covered with absorbent paper during transport to the incubator.

During this incubation, the 32P will be taken into the cells and incorporated into ATP. Continue the incubation until a steady state is reached. Empirically, the time to steady state is cell-type dependent and is determined as that length of time where radiolabeling of a sample phosphoprotein is not increased by lengthening the incubation time.

3. Carry out any manipulation of the cells required in the experiment you have designed. To do so, pipet the 32P-labeled cells up-and-down to suspend and then aliquot into 15-ml screw-cap tubes. Cap tightly and incubate for the desired length of time.
Frequently, in a phosphorylation experiment, unstimulated cells are compared to cells stimulated through a cell-surface receptor or stimulated pharmacologically by reagents that activate protein kinases (see UNIT 3.12; however, doses may need to be adjusted). For example, phorbol esters may be used to activate protein kinase C, and agents that alter cellular cAMP levels (dibutyryl cAMP or forskolin) may be used to activate protein kinase A.

To avoid excessive exposure to radioactivity, it is helpful to add ligands for stimulation to tubes first. Stimulation can be carried out in a shaking water bath to prevent pelleting of cells.

4. Stop the incubation by diluting five- to ten-fold with ice-cold PBS/PI (to dilute radioactivity).

Centrifuge the cells 5 min at 500 × g, 4°C. Pour off the radioactive supernatant directly into a well-shielded liquid radioactive waste container (to remove unincorporated label). Keep the cells on ice for the remainder of the protocol.
To avoid contamination, use a waste container with a wide mouth. Hold a Kimwipe below the opening of the tube to catch any drips.
Comments:

CAUTION: It is extremely important to follow safety precautions when performing [32P] orthophosphate labeling, as it frequently requires the use of large amounts of 32P (often ≥10 mCi at a time). Spilling even a tiny droplet of radioactivity must be strictly avoided and complete adherence to laboratory regulations concerning the use of β-emitting radioactivity is required. In addition, the following measures are quite useful.

For protection of the investigator, disposable lab clothing—including disposable lab coat, sleeves, booties and double gloves—is indispensable. Keep a Geiger counter turned on nearby whenever working with the radiolabel and monitor your gloves, your clothing, and the environment frequently. Set up a protected area to work behind with lucite screens on all sides.

For protection of the environment, cover the floor around the work area with "diapers" (plastic-backed absorbent paper) to absorb any inadvertent spills. When transporting cells with radiolabel from the lab bench to the incubator, have cells behind a lucite screen on absorbent paper inside a flat basin. Monitor all areas where radiolabel was used immediately afterwards, including the lab bench, centrifuge, incubator, and shaker water bath.

Some special containment equipment is useful. The liquid radioactive waste container should be shielded to provide adequate protection. We enclose the plastic carboy in a box made of Ã¢â€¦Å“ -in.-thick Plexiglass. The local radiation safety officer can assess if shielding is adequate. When removing the label from the source vial, it is helpful to enclose the vial in a vial shield. These are available made of -in.-clear Plexiglass with a stabilizing side-thumb screw to hold the vial in position.

Record keeping concerning the use of the radiolabel is essential. A central book for recording all usage of [32P]orthophosphate should be employed. In addition, because contamination and excessive exposure often occur when individuals inadvertently fail to follow one of the rules mentioned above, filling out a standardized check-off sheet is important. Such a sheet should document the use of

proper disposable clothing and shielding, protection of the floor with diapers, and a Geiger-counter survey of all areas where 32P was used.