Labeling cells with 3H -inositol

Material:
1) inositol free media (from ICN DMEM cat#2642954)
2) glutamine 100x
3) 3H inositol from ARC inc (1-800-331-6661) cat#ART116
4) Plate 100mm
5) dialyzed serum

Labeling:

1) Rinse the cells with PBS and incubate them with inositol free media for about 30 min (pre-labeling).

2) add fresh inositol free media (5 ml / P100) containing :
glutamine
dialyzed serum (0.5% for serum starved cells or 10% for exponentially growing cells)
10 microCurie of 3H inositol per ml (The inositol comes in ethanol. If you think that the final concentration of ethanol may affect your cells, you can choose to evaporate it in an eppendorf tube and ressuspend it in the inositol free media)

3) Label the cells for 24-72 hs (48 hrs is ideal).

4) rinse the cells in 5 ml PBS. After removing the PBS, keep the plate tilted for a few seconds to remove the last drop of PBS with a P1000 pipet tip (important!)l

5) Add 400µl of 1M HCl and then add methanol 400µl.

5) scrape the cells and transfer to an epp tube using a P1000 pipet tip clipped at the end (because the lysate will have a lot of white insoluble material).

OPTIONAL: add carrier lipids to the tube where the lysate is being transferred to. This is especially important if you are starting with few cells. You can use crude brain phosphoinositides from Sigma or a mixture of any lipids you have.

6) Add 400µl chloroform, vortex **very well**, spin (approx. 1 min in a bench top centrifuge at 14,000 rpm. If using nanofuge, spin them longer) and collect bottom phase (organic phase) into a new epp tube. The white insoluble material should make a thin line on the interface. Avoid this material when collecting the lipids.

7) To each tube containing the organic phase, add 400 µl of a mixture of freshly prepared Methanol:0.1M EDTA pH8.0 (1:0.9, v:v). Vortex very well, spin and collect the bottom phase into a new epp. tube.

* At this point you can estimate the total counts obtained by measuring the cpm present in 1 µl of each sample using a scintillation counter. You should have at least 1,000,000 cpm total per sample.

8) evaporate the organic phase containing your lipids under a Nitrogen stream.

Store this extract at -70 ^{o}C until deacylation..

TIPS FOR AVOIDING CONTAMINATION OF THE AREA: use good quality epp tube. Some brands don't seal well and will leak during vortexing. When vortexing radioactive organic solvents, use a kimwipe around the tube to avoid spraying radioactivity around. Always spin the tubes before opening the cap. When pipeting organic solvents, saturate the pipet tip first, to avoid dripping. When transferring the chloroform from one tube to another, hold both tubes in one hand to avoid having to move the pipet tip through a long distance. Wipe test the area after done.