

DRYING DOWN ^{32}P -LABELED NUCLEOTIDES FROM MIXTURES OF ETHANOL AND WATER

Most commercial suppliers sell [^{32}P]dNTPs as concentrated, stabilized aqueous solutions that can be added directly to the appropriate reaction mixtures. However, some manufacturers still supply [^{32}P]dNTPs dissolved in 50% ethanol, which must be removed by evaporation before the [^{32}P]dNTP can be used.

1. Using an automatic micropipettor, carefully dispense the desired quantity of [^{32}P]dNTPs into an Eppendorf tube.
2. Plug the top of the tube with a small piece of cotton and cover with two or three layers of parafilm (Fig. A.1).
3. Poke many holes in the parafilm with a needle.
4. Place the tube securely in a beaker or rack and evaporate the [^{32}P]dNTPs to dryness under vacuum at room temperature or in a lyophilizer.
5. Discard the parafilm and cotton into radioactive waste. Add a small volume (5 μl) of H_2O to the tube. Vortex for 15 seconds.
6. Add the remaining ingredients of the reaction mixture to the tube. Mix by vortexing and incubate as indicated in the relevant protocol.

Notes

- i. Steps 1-3 can be eliminated by using a speed-vac concentrator, which prevents bumping of the contents of the tube under vacuum.
- ii. Wherever possible, manipulations involving ^{32}P should be carried out behind lucite screens to shield personnel from exposure to radioactivity.

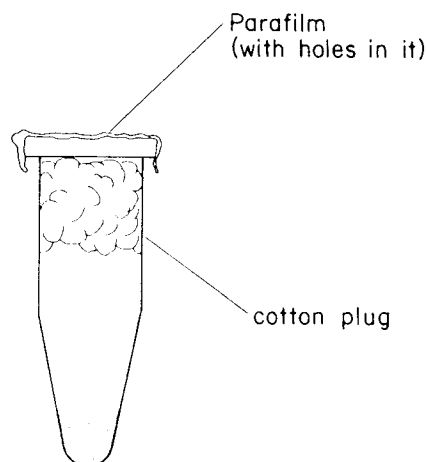


Figure A.1