Nucleotide Extraction From Cells

MATERIALS

- Diethyl ether (Sigma, 346136)

Preparation of Water Saturated Ether:

- Add 50 mL of Milli-Q water to 500 mL of diethyl ether and mix. The upper layer is the water saturated ether.

PROCEDURE:

1. To a fresh cell pellet add 400 µL of 10% TCA.

2. Using the pipettor mix the cells up and down 10 times and place them on ice for 10 min.

3. Again mix the cells up and down 10 times and then centrifuge at 12000g at 4 ^{o}C for 15 min.

4. Transfer the supernatant (contains the nucleotides) to a new 2 mL centrifuge tube.

5. Add 1200 µL of water saturated ether and pipet up and down carefully until the two solutions are thoroughly mixed. Keep the tube on ice during this process.

NOTE: This step is required to increase the pH of the TCA extract.

6. Centrifuge the sample at 12000g for 5 min at 4 ^{o}C and then carefully remove and discard the top layer.

NOTE: The nucleotides will be in the bottom layer.

7. Repeat steps 6 and 7 a total of three times. After the last extraction leave the tube open, on ice, for 10 min to allow the ether to evaporate.

8. Next freeze the extract and then lyophilize to dryness.

9. The dried material can be resuspended and analyzed by HPLC for composition analysis.

CITATION:

Fairbanks L, Jacomelli G, Micheli V, Slade T, Simmonds H; Biochem. J. (2002) 366, 265-272