

Flow-Chart Example

Purification of Lactate Dehydrogenase (LDH)

Mince 8 g of meat with scissors and put it into a high speed blender together with 50 mL of cold 50 mM potassium phosphate, pH 7.2. Homogenize the meat at top speed for a total of 2 min. Pour the homogenate into two 50 mL round-bottom plastic centrifuge tubes. Using a transfer pipet and a double beam balance, adjust the distribution of homogenate between the two tubes so that they are the same weight. Centrifuge the tubes at 4 °C for 5 min at 17,000 rpm.

Collect the supernatant immediately by decanting into a 50 mL graduated cylinder through a funnel containing loosely packed glass wool. This removes the clumps of lipid. Protect your hands with gloves when working with glass wool. Discard the pellet into the trash. The supernatant is the crude extract. Record its volume. Transfer 1 mL of the crude extract to a 15 mL conical plastic tube and keep the tube in a test tube rack. The test tube rack should be kept in an ice bath to keep the samples cold. Small aliquots of this will be used for carrying out the enzyme activity and protein assays from which you will calculate the amount and specific activity of LDH in your crude extract.

Transfer the crude extract to a 100 mL beaker containing a magnetic stirring bar in preparation for ammonium sulfate fractionation. Ammonium sulfate precipitation of proteins is most effective in the cold. Prepare a small ice bath in a crystallizing dish and place it on a magnetic stirring plate. Put the 100 mL beaker containing the crude extract into the ice-bath and begin stirring. Weigh out 0.24 g of ammonium sulfate for each mL of crude extract present. Add the ammonium sulfate slowly to the crude extract.

Flow-Chart for Isolation of LDH

