

Protein extraction from animal and plant source

Experiment (1): Protease inhibitor extraction from plant source

Materials:

Chemical

Plant tissue, phosphate buffer 0.1 M (pH 7.0), distal water.

Equipment and Glassware

Measuring, centrifuge tube, measuring cylinder, cheesecloth, shaker, blade, blender, electronic balance, centrifuge.

Protocol:

1. Weight 12 g of the sample and place it in the blender with 200 ml of the extraction buffer (phosphate buffer 0.1 M, pH 7.0).
2. Incubate the homogenate at room temperature on a rotary shaker for 30 min at 150 rpm.
3. Filter the slurry through cheesecloth and then transfer to centrifuge tube.
4. Centrifuge the filtrate at 10,000 rpm for 10 min at 4°C for the removal of any cell debris that remained in the preparation.
5. Measure the volume of the supernatant.

Results:

Volume of the supernatant (crude extract) = _____ ml

Experiment (2): Lactate dehydrogenase extraction from animal source

Materials:

Chemical

Animal tissue, 0.1 M Tris-HCl (pH 7.4), distal water.

Equipment and Glassware

Measuring cylinder, blade, blender, electronic balance, centrifuge.

Protocol:

1. Cut ~7.5 g of muscle tissue from the tissue source (*Record the exact weight of tissue used*).
2. Cut the tissue into small pieces, discard the connective tissue and fat.
3. Add 38 ml of cold extraction buffer (0.1 M Tris-HCl, pH 7.4) in a blender with the sample. note: (20% weight/volume).
4. Transfer the homogenized tissue/buffer mixture into centrifuge tubes. *Note: balance the tubes.*
5. Centrifuge your homogenate for 5 minutes at 7,000 rpm.
6. Measure the volume of the supernatant.

Results:

Volume of the supernatant (crude extract) = _____ ml