Protein isolation and troubleshooting

By

Alaa El -Din Hamid Sayed,

Assistant lecturer at Zoology Department, Faculty of Science, Assiut Uinversity

Alaa_h254@yahoo.com

Selection of a protein source

Serum

cell cultures (bacteria, yeast, mammalian, etc.)

Tissue

genetically engineered - tagged proteins, over-expression

Liquid (hemolymph, milk)

Plant leave

Plant seed

must be fresh or at least reserved at -80ºC

Extraction and Analysis of Diagnostically Useful Proteins from Formalin-fixed, Paraffin-embedded Tissue Sections (Morito etal 1998)

Composition of lysing buffer:

100ml 0.625M (0.706 g/100ml) Tris-HCl (pH 6.8) – 2% SDS (w/v) - 10% glycerol (v/v) - 5% 2-mercaptoethanol (v/v)

Isolation of protein procedures

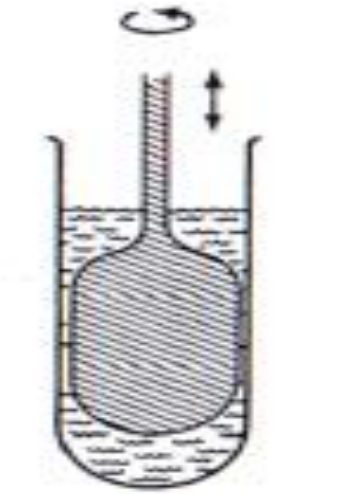
first method

(~o.1 gm fresh weight) of the sample was suspended in 1.0 ml lysing buffer. Heated at 100 ˚C for 5 min., centrifuged at 10,000 rpm for 30 min., and 100 µl of each supernatant as extracted protein used for protein analysis.

the second method

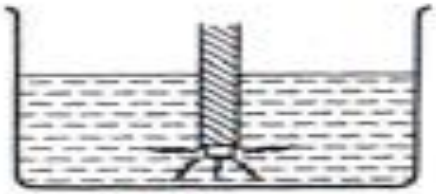
1. Label all tubes. Prepare solutions and have ready at hand.
2. Remove the tissue from the –80oC freezer and thaw on ice. If the tissue is fresh, keep on ice (or alternatively work in a cold room).
3. Place tissue in a mortar and pestle.
4. Add ~ 1ml of lysing buffer per ~0.1g tissue.
5. Grind tissue until no more chunks are visible.
6. Remove ~1ml of the liquid grindate into a microfuge tube.

7. Place on ice.
8. Rinse mortar and pestle to remove all traces of sample and proceed to the protein isolation of the next tissue sample.
9. Spin samples at top speed in the microfuge
10. Transfer the liquid supernatant into a second (new) microfuge tube.
11. Store samples in the –80ºC.

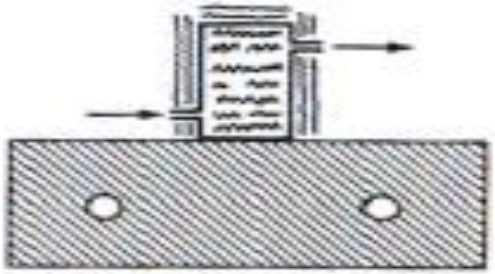


(a) Hand-operated or motor-driven

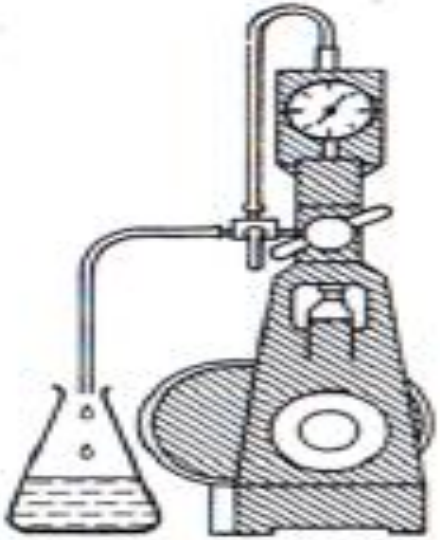
(b) Waring blender



(c) Ultrasound



(d) Vibrating bead mill



(e) Manton-Gaulin homogenizer

Figure 2.2. Equipment used for breaking up cells to obtain an extract. (a) Hand-operated or motor-driven glass homogenizer. (b) Waring blade-blender (food processor). (c) Ultrasonic probe. (d) Vibrating glass bead mill. (e) Manton-Gaulin cell disintegrator.

Troubleshooting

1- Chemical expiry

2- Temperature, PH

3-Lysis of sample

4- Protenase enzyme

5- Contamination (bacteria, fungi, etc.)

To Be Right You Should Measure Total Protein Using Spectrophotometer


Questions