CONCEPT: DIFFERENTIAL CENTRIFUGATION

●*Centrifugation*: process that uses _____ & centrifugal force to separate particles in a mixed solution.

    □ Insoluble particles form solids (or _____), are pulled down faster & form a _____ at the bottom.

    □ _____: left-over solution above the pellet with more soluble unprecipitated solutes.

●*Sedimentation coefficient* (Svedberg = S): characterizes _____ of sedimentation & particle behavior in a centrifuge.

    □ The greater the S value, the _____ the movement of the particle to the bottom to form a pellet.

    □ S depends on particle & solvent properties (ex. _____, shape & _____).

EXAMPLE: Centrifugation.
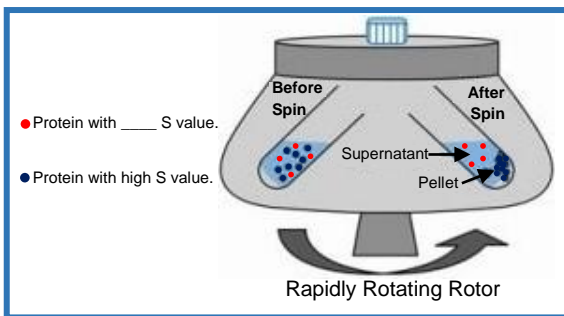
| Protein	S value (Svedberg Units)	Molecular Weight (g/mol)
Pancreatic trypsin inhibitor	1	6,520
Cytochrome c	1.83	12,310
Ribonuclease A	1.78	13,690
Myoglobin	1.97	17,800
Trypsin	2.5	23,200
Carbonic anhydrase	3.23	28,800
Concanavalin A	3.8	51,260
Malate dehydrogenase	5.76	74,900
Lactate dehydrogenase	7.54	146,200

PRACTICE: Which of the following affects the sedimentation of a particle during centrifugation?

a) Mass.   b) Shape.   c) Density of the particle & solvent.   d) a & b.   e) a, b & c.

2) Differential Centrifugation

●After cells are homogenized, the crude extract can be subject to _____ centrifugation.

    □ *Differential Centrifugation*: _____-wise centrifugal separation of organelles using precise spinning velocities.

EXAMPLE: Differential Centrifugation.
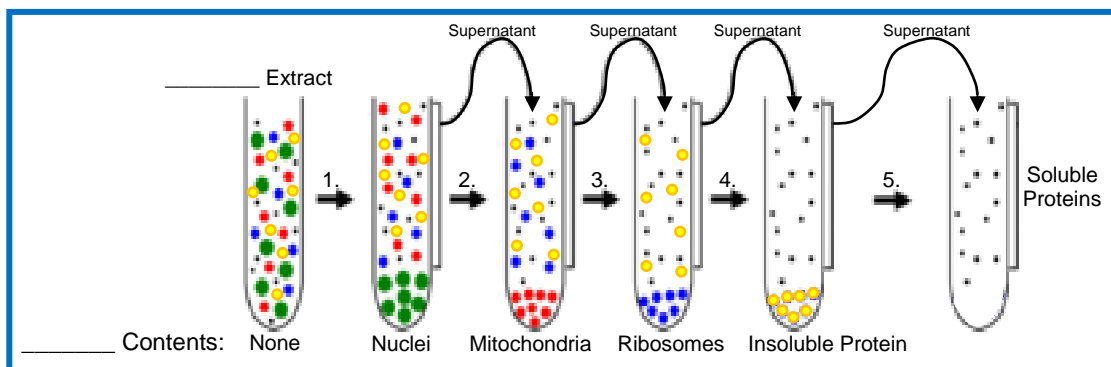
PRACTICE: What is the main purpose of differential centrifugation?

a) To make the cells dizzy before purifying proteins.   c) To pellet all unwanted proteins.

b) To separate out fractions of cell components with similar S values.   d) To fully purify the target protein of interest.