Examination of protein purity

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What is next after purifying protein?

1. The purity of the protein must be checked. How?
2. The molecular weight MW of the protein must be analyzed and conformed at the expected size. How?
3. The buffer solution needs to be changed sometimes. How?
4. The protein concentration needs to be measured. How?
5. The low concentration protein needs to be concentrated. How?
6. Finally, the protein needs to be frozen and stored at -20 to -80 ˚C. How?

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How to analyze the purity of the protein?

- Using Gel-electrophoresis SDS-PAGE method.
- What is the SDS method for?
- SDS-PAGE is an analytical technique to separate proteins based on their molecular weight
- What is based on?
- The protein migrates by applying an electric field allowing the protein to pass through the pores inside the matrix (gels).
- Why it is called SDS PAGE?
- Because the chemical ''SDS'' is used to denture the protein and allow it to pass through the gels holes.

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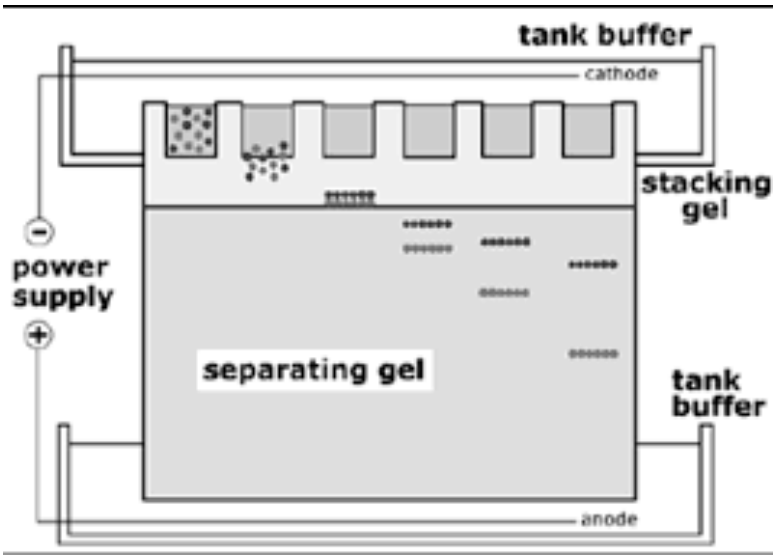
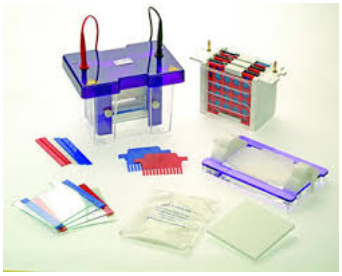
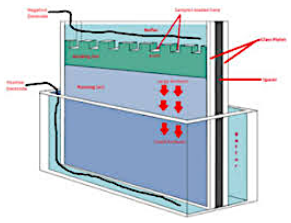
SDS page and equipment







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Describe the SDS-page?

1. The full SDS page contains two gels layer.
2. The large gel called separating gel.
3. The small gel called staking gel.
4. The full page then immerged in a glycine buffer.
5. An electrical field is applied for migration.
6. The large protein will stop first, the small protein will take longer.
7. A protein marker is used to analyze the gel.
8. The gel is the then stained by a dye to show the band of the protein.

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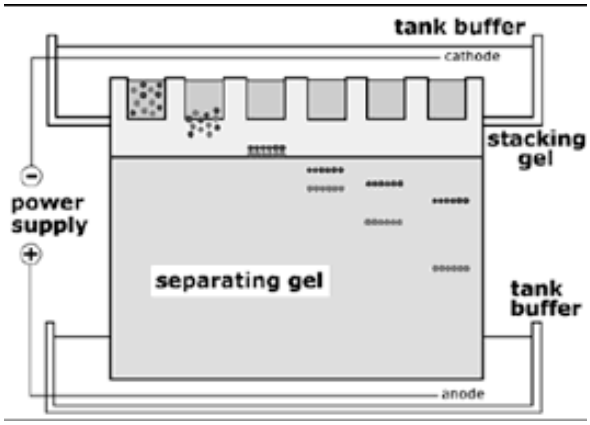
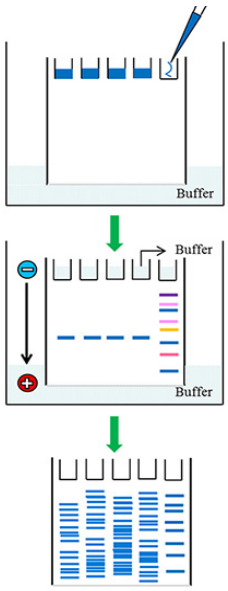
The Electrical migration process?





Protein samples and marker loaded in vertical SDS-PAGE system

Direction of migration of samples in vertical SDS-PAGE system

SDS-PAGE gel after Coomassie blue staining

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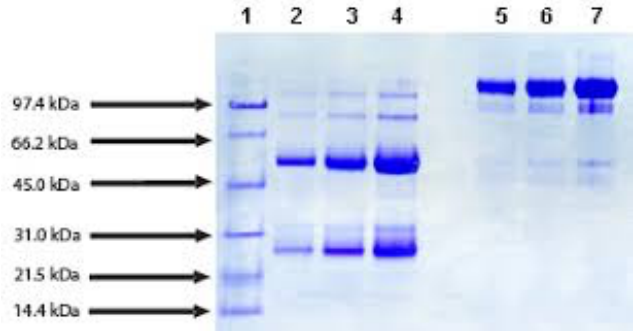
Describe this SDS page ? The MW?

1. Is the protein pure?
2. How many bands in the single lane?
3. Is it one protein in a lane or more? complex?
4. What is the purity % of the protein?
5. What is the MW of each band?



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What is the Dialysis?

Dialysis step allows for:

1. buffer exchange.
2. removal of salts or other contaminants to the protein samples.
3. It is typically carried out using various forms of semi-permeable membranes and tubing.
4. Due to the pore size of the membrane, large molecules in the sample cannot pass through the membrane.
5. Each tube has special MWCO (molecular weight cut off) !! Which must be less than half of the MW of the protein in order to avoid loosing protein.

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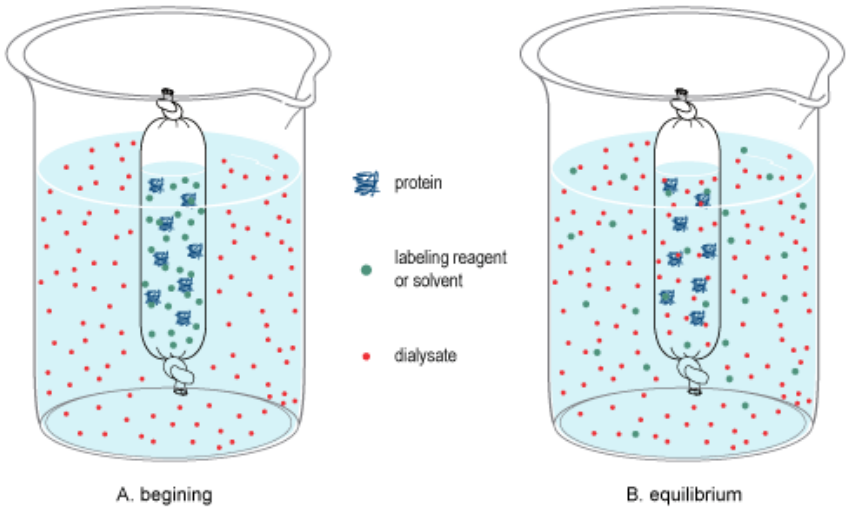
Dialysis



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How do you measure the protein concentration?

- There are several methods to measure the Conc.
- Measure the Absorption A or called Optical density O.D using spectrophotometer at 280 nm wavelength.
- In this method, we need to know:
- The molar absorptivity E of the protein by calculating the number of aromatic amino acids in the protein which are Tryptophan and Tyrosine (in the websites). Why?
- Because the aromatic ring gives absorption at 280 nm.

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So, what ?

- So, what does that mean?
- Means: from Beer-lambert equation which is (A=E x b x c) we can calculate the concentration.
- A= absorption.
- E= molar absorptivity.
- b= cuvette width usually 1 cm.
- C=the concentration

- For example:
- If the molar absorptivity protein = 0.25 g/L
- And we read the O.D (optical density) e.g. A=1
- (A=E x b x c) 1= 0.25 x1 xc
- c=4 mg/ml

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What about if the protein in a buffer is diluted?

- The protein buffer needs to concentrate.
- There are several ways to concentrate protein:

1. lyophilization method:
2. precipitation by Ammonium sulphate.
3. Using filter tubes and others.

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Lyophilization method:

- Describe the lyophilization method?
- It is a method used for concentrating and powdering proteins.
- What should I do to protein before lyophilization.
- The protein must be flash frozen by liquid N2
- How does it work?
- It works by a sublimation in the freeze-drying process.

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Precipitation by Ammonium sulphate.

- Describe concentrating protein by NH_2SO_4 ?
- it stabilizes proteins.
- It precipitates the proteins by salting out at high ionic strength. Thus, the protein will be concentrated.
- Protein could be re-solubilized in a buffer and then dialyzed multiple times. Why? To get red of the salts.

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How do you store the protein?

- Protein must be frozen by Liquid N2 ?
- Why?
- Because it flashes freeze the protein very quickly which avoids the degradation in the structure.
- Usually, protein stored for a short time at -20 ˚C.
- But for long time, it must be stored at -80 ˚C.

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