FAQs

1.What is electrophoresis?
Electrophoresis is the migration of charged molecules in an electric field toward the electrode of opposite charge.
2.What are some uses for electrophoresis?
Uses of electrophoresis include determining the purity of a protein sample, the amount of DNA in a sample, the size of a protein, and the nucleotide sequence of a DNA sample.
3.What is a cathode? …an anode?
cathode – negative electrode anode – positive electrode
4.How is the amount of sieving done by a gel controlled?
Sieving is controlled by changing the percentage (%) of agarose or acrylamide in a gel

5.What would determine whether agarose or acrylamide matrix should be used?
The size of the molecules being separated determines the matrix to be used (e.g., >200 kDa use agarose).
6.What are the major functions of acrylamide gel electrophoresis?
The major functions of acrylamide gel electrophoresis are separation of most proteins and electrophoresis of small DNA molecules.
7.What is charge density?
Charge density is the ratio of charge to mass.
8.How does charge density relate to electrophoresis?
Charge density relates to electrophoresis because proteins can be separated according to their charge density since both their charge and mass will affect their migration. Often, however, charge is removed as a factor and only mass will determine rate of migration.
9.What step is taken to do molecular weight determinations using PAGE?
In order to determine molecular weights using PAGE, charge must be removed as a factor. SDS is put in the gel buffer and in the sample buffer to give the proteins an overall negative charge. SDS also gives all proteins identical charge to mass ratios, thus removing charge as a factor in migration.
10. What does SDS-PAGE stand for?
SDS-PAGE stands for sodium dodecyl sulfate polyacryamide gel electrophoresis.
11. What is the function of SDS?
The function of SDS is to remove charge as a factor, so the molecular weight of a protein can be determined.
12. What is native PAGE?
Native PAGE refers to running a protein in its natural state without breaking bonds or removing charge as a factor.
13. When is native PAGE used?
Native PAGE is used when the structure and activity of a protein need to be preserved.
14. What is done to get the proteins to enter the gel at the same time?
To get proteins to enter the gel at the same time, a stacking gel is poured on top of the separating gel.

15. What is a stacking gel? …a separating gel?
A stacking gel is a gel that is poured on top of the separating gel and is of a much lower acrylamide concentration and a lower pH than the separating gel. Its function is to allow the proteins to enter the separating gel at the same time. The separating gel is used to separate the proteins according to molecular weight.
16. Why should precautions be taken when making up a polyacrylamide gel?
Acrylamide is a neurotoxin in its unpolymerized state (before the gel solidifies).
17. When is acrylamide safe to handle?
Acrylamide is safe to handle when it is polymerized (in gel form).
18. Why does this lab use a separating gel of 15% acrylamide?
A 15% acrylamide gel provides good separation for proteins in the range of 14 to 100 kDa.
19. What are the differences in the way an agarose and an acrylamide gel are run?
An agarose gel is run in a horizontal chamber and the gel is completely surrounded by buffer. An acrylamide gel is run in a vertical chamber and has two separate buffer chambers. One chamber allows buffer to be in contact with the upper portion of the gel and the other chamber with the lower portion of the gel.
20. What function does heating serve in preparing a protein for electrophoresis?
Heating in the presence of SDS denatures the proteins, allowing them to become linear for running in the gel.