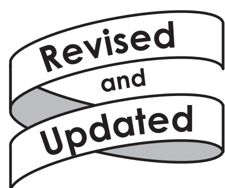
The Biotechnology Education Company ®



255
EDVO-Kit #

Purification & Size Determination of Green (gfp) & Blue (bfp) Fluorescent Proteins

Storage:
Some components require freezer storage.
See page 3 for specific storage instructions.

EXPERIMENT OBJECTIVES:

Students will learn to partially purify the Green (gfp) and Blue (bfp) fluorescent proteins. In an optional activity, the molecular weights of the proteins will be determined.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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EVT 2011_12_02AM

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There is enough of each sample for six (6) groups sharing three polyacrylamide gels.

Store components A-D, F, G in the freezer.

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EXPERIMENT COMPONENTS

		Storage
A	Cell Extract containing green protein (gfp)	Freezer
B	Cell Extract containing blue protein (bfp)	Freezer
C	Column Elution Buffer (10x)	Freezer
D	Standard Protein Markers	Freezer
E	Dry Matrix	Room temperature
F	Protein Denaturing Solution	Freezer
G	50% Glycerol Solution	Freezer
•	Tris Glycine SDS Electrophoresis Buffer (10x)	Room temperature
•	Protein InstaStain® sheets	Room temperature
•	Chromatography Columns	Room temperature

None of the components have been prepared from human sources.

REQUIREMENTS

- Vertical Gel Electrophoresis Apparatus (optional)
- D.C. Power Source (optional)
- Automatic Micropipet (5 -50µl) and Tips
  (Cat. #638 Fine Tip Micropipet Tips recommended)
- Balance
- Ice Buckets and Ice
- Long U.V. lamps (Cat. #969)
- Ring stands and column clamps
- 1ml pipets and pipet pumps
- Microtest tubes
- Distilled or deionized water
- Three Polyacrylamide gels* (OPTIONAL)
- Glacial acetic acid (OPTIONAL)
- Methanol (OPTIONAL)

*Polyacrylamide gels are not included for the electrophoresis part of this experiment. The experiment is designed for two student groups to share a gel. A total of 3 polyacrylamide gels (Precast polyacrylamide gels, Cat. #651) are required.

Background Information

PURIFICATION & SIZE DETERMINATION OF GREEN & BLUE FLUORESCENT PROTEINS

Bioluminescence from marine microorganisms has been observed by many summer visitors at various beaches around the world. It always fascinates the observer by the repeated parade of both color and light on the sand during the ebb and flow. This observation takes second place to the light produced by the bioluminescent jelly fish, Aquorea victoria. A bright bursting energy of light is observed when energy is transferred to the green fluorescent protein (gfp) which is located in a specialized photogenic cell located in the base of the jellyfish umbrella. There are several variants to gfp protein that have been genetically engineered and which dramatically enhance classroom laboratory experiments. An excellent companion to gfp is the blue fluorescent protein (bfp) which is cloned and well characterized.

This family of proteins had been known for some time and significant research in this area had been published. The fluorescent proteins have been cloned and expressed. These proteins do not require substrates, other gene products, or cofactors. When exposed to long or short U.V. light, they will emit a bright green or blue light that is clearly visible in bacteria that are transformed by plasmids that contain genes for the gfp or bfp. Likewise, purification of gfp or bfp is simplified by their detection based on fluorescence.

There are many examples of chimeric proteins that are fusion products using the gfp or bfp fluorescent proteins as biological tags. Such fusions are at either the N- or C- terminal and often result in no loss in the fluorescence or biological activity of the chimeric protein. These new biotechnology tools have made it possible to conduct studies that deal with protein localization and trafficking within cells.

The green fluorescent protein (gfp) has 238 amino acid residues and has a molecular weight of approximately 40,000 daltons. It appears that most of the intact protein is required for maintaining fluorescence and only small deletions of a few amino acids are allowed without compromising the integrity of the protein structure. Interestingly, the chromophore responsible for light emission is within the primary structure of the protein and resides in a tripeptide at positions 65 to 67 which is cyclic and is composed of the amino acids Ser-Tyr-Gly. The importance of protein folding is clearly demonstrated with gfp where the protein is fluorescent only upon proper conformational folding.

The blue fluorescent protein (bfp) is a derivative variant of the gfp. It has a His-66 substitution at the Tyr-66 position and a second substitution from Tyr-145 to Phe-145. The initial bfp known as P-4 had only the His-66 substitution and was not as bright as the double mutant. With the crystal structure of gfp determined, several other variations of the gfp were genetically engineered using site directed mutagenesis. In such procedures, specific mutations are introduced in a protein to determine the impact of the mutation on structure and function of the protein. The set of gfp and bfp proteins can be used as a dramatic tool to visually demonstrate the effect of pivotal amino acid changes on the structure and function of proteins. Amino acid substitution can also be used to demonstrate the effects of accumulated mutations on aging and various cancers.

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Background Information

In this experiment, bacterial extracts containing gfp or bfp will be fractionated by chromatography using a molecular sieve matrix. Factors that affect the separation include size, shape and associated non-protein biologicals such as carbohydrate residues. The fluorescent proteins will be detected on the column and subsequently in the test tubes by examination under long U.V. light. For most proteins, such columns can also be used to determine apparent molecular weights. Accurate estimation of protein polypeptide composition and size(s) are performed by analyzing the fractions that contain the protein of interest by denaturing polyacrylamide gel electrophoresis.

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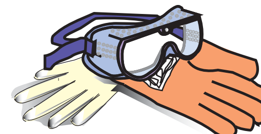
Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will learn methods and procedures to partially purify the FluoroGreen™ (gfp) and FluoroBlue™ (bfp) fluorescent proteins. The molecular weights of the proteins will be determined using denatured SDS polyacrylamide gel electrophoresis.



LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

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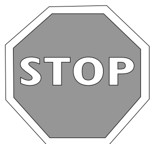
Packing and Equilibrating the Column

The loading of the column and subsequent elution will be done at room temperature. The elution buffers and the fractions collected will be stored on ice as they elute from the column.

1. Vertically mount the column on a ring stand. Make sure it is straight.
2. Slide the cap onto the spout at the bottom of the column. Fill about one-third of the column with the elution buffer.
3. Mix the slurry (molecular sieve) thoroughly by swirling or gently stirring.
4. Carefully pipet the mixed slurry into the column by letting it stream down the inside walls of the column.

   If the flow is stopped by an air pocket, stop adding the slurry and firmly tap the column until the air is removed and the slurry continues to flow down the side of the column.
5. Place an empty beaker under the column to collect wash buffer.
6. Remove the cap from the bottom of the column and allow the matrix to pack into the column.
7. Wash the packed column with 5ml of 1x elution buffer. Do not allow the column to dry.
8. Slide the cap onto the spout and make sure it does not drip.



OPTIONAL STOPPING POINT

The prepared DNA sample can be stored in the freezer for electrophoresis at a later time.

Remember!

Do not allow the column to dry.

NOTE:

The loading of the column and subsequent elution will be done at room temperature. The elution buffers and the fractions collected will be stored on ice as they elute from the column.

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Partial Purification of Green & Blue Fluroescent Proteins

COLLECTING COLUMN FRACTIONS OF (gfp) PROTEIN

1. Label the first set of eight microtest tubes #1-8.

2. Slowly load the column with 0.2ml of the gfp extract. Allow the extract to completely enter the column.

3. Elute the column with 1x elution buffer.
   - Add buffer slowly (several drops at a time) to avoid diluting the protein sample.
   - Using the graduated marks on the sides of the tubes, collect 0.5ml fractions in the labeled microcentrifuge tubes.
   - Store fractions **on ice** immediately upon collection.

*** * * Remember - Do not allow the column to dry. * * ***

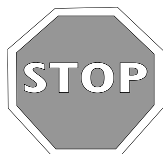
4. Check all fractions by using long wave U.V. light to identify tubes that contain the fluorescent gfp or bfp proteins.

5. Save the fractions containing gfp proteins for further analysis by SDS gel electrophoresis (optional).

COLLECTING COLUMN FRACTIONS OF (bfp) PROTEIN

1. Wash the column with 10ml of 1x Elution buffer.

2. Label a second set of eight microtest tubes #9-16. Repeat steps 2-5 (from above) with 0.2ml of the (bfp) extract. Store the 0.5ml fractions **on ice** immediately upon collection. **Do not allow the column to dry.**



OPTIONAL STOPPING POINT

If time does not permit you to continue with the SDS polyacrylamide analysis, you may freeze the fractions at -20°C and perform the assays at a later date.

NOTE:

If you shine long U.V. light (black light) on the column, you will see (gfp) or (bfp) migrating through the column and you can predict the peak tubes that will contain the protein.

DO NOT USE SHORT U.V. LIGHT (USED FOR DNA WORK). DIRECT VISION WILL CAUSE BURNS AND DAMAGE TO EYES!

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Sample Preparation for Denaturing SDS-Gel Electrophoresis (OPTIONAL)

1. Identify the tube with the highest fluorescence of the gfp. Transfer 200µl each of the peak extract into two clean microtest tubes. Label one tube "gfp native" and the second tube "gfp denatured".

2. Identify the tube with the highest fluorescence of the bfp. Transfer 200µl each of the peak extract into two clean microtest tubes. Label one tube "bfp native" and the second tube "bfp denatured".

PREPARING NATIVE PROTEINS (UNBOILED)

The protein in its native form can be shown to fluoresce with a long wave U.V. light.

3. Add 25µl of 50% glycerol (G) to each of the tubes labeled "gfp native" and "bfp native".

4. Mix and set these tubes aside for later electrophoresis.

Quick Reference

Proteins unfold and lose their tertiary structures by boiling for 5 minutes in the presence of denaturing solutions which contain SDS and 2-mercaptoethanol. In the absence of boiling, regions of the protein can remain intact in their native state.

PREPARING DENATURED PROTEINS (BOILED)

Note: Denaturing the proteins will remove their fluorescence.

5. To denature the protein samples, add 25µl of protein denaturing solution (F) to each of the tubes labeled "gfp denatured" and "bfp denatured". The denaturing solution contains sodium dodecylsulfate (SDS) and 2-mercaptoethanol.

6. Bring a beaker of water, covered with aluminum foil, to a boil.

7. Make sure the sample tubes to be denatured (boiled) are tightly capped and thawed.

8. The bottom of the tubes should be pushed through the foil and immersed in the boiling water for 5 minutes. The tubes should be kept suspended by the foil.

9. Allow the tubes to cool for a few minutes at room temperature.

10. Proceed with Electrophoresis of Proteins as outline on page 10.

DENATURING THE STANDARD PROTEIN MARKERS

- If your standard protein marker has not been rehydrated and boiled by your instructor, add 130 µl of distilled or deionized water to it and allow the sample to rehydrate for several minutes. Vortex or mix vigorously. Then, proceed to denaturing the standard protein markers as outlined in steps 6-9 above.

- If your standard protein marker has already been denatured, proceed with Electrophoresis of Proteins as outlined on page 10.

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Electrophoresis of Proteins (OPTIONAL)

PREPARING THE POLYACRYLAMIDE GEL FOR ELECTROPHORESIS

Precast Polyacrylamide Gels:

Precast polyacrylamide gels will vary slightly in design. Procedures for their use will be similar.

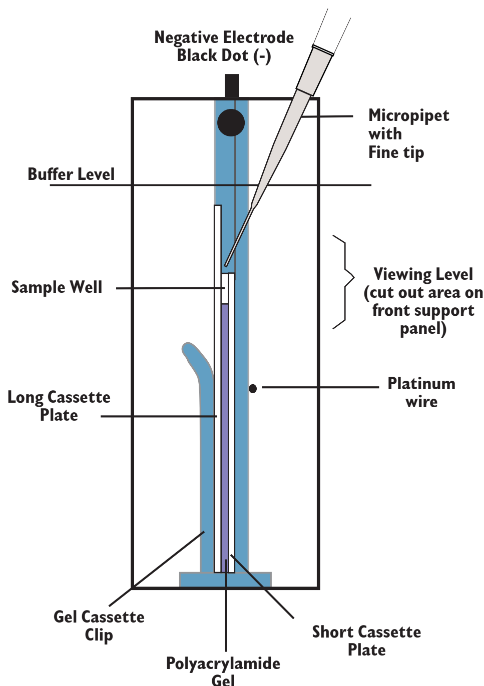
1. Open the pouch containing the gel cassette with scissors. Remove the cassette and place it on the bench top with the front facing up.

   Note: The front plate is smaller (shorter) than the back plate.

2. Some cassettes will have tape at the bottom of the front plate. Remove all of the tape to expose the bottom of the gel to allow electrical contact.

3. Insert the Gel Cassette into the electrophoresis chamber.

4. Remove the comb by placing your thumbs on the ridges and pushing (pressing) upwards, carefully and slowly.



The figure above shows a polyacrylamide gel cassette in the EDVOTEK® Vertical Electrophoresis Apparatus, Model #MV10.

PROPER ORIENTATION OF THE GEL IN THE ELECTROPHORESIS UNIT

1. Place the gel cassette in the electrophoresis unit in the proper orientation. Protein samples will not separate in the gel if the cassette is not oriented correctly. Follow the directions accompanying the specific apparatus.

2. Add the diluted buffer into the chamber. The sample wells and the back plate of the gel cassette should be submerged under buffer.

3. Rinse each well by squirting electrophoresis buffer into the wells using a transfer pipet.

   The gel is now ready for practice gel loading or sample loading.

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Electrophoresis of Proteins (OPTIONAL)

PRACTICE GEL LOADING

EDVOTEK® Cat. #638, Fine Tip Micropipet Tips are recommended for loading samples into polyacrylamide gels. A regular microtip may damage the cassette and result in the loss of protein samples.

1. Place a fresh fine tip on the micropipet. Aspirate 20 µl of practice gel loading solution.

2. Place the lower portion of the fine pipet tip between the two glass plates, below the surface of the electrode buffer, directly over a sample well. The tip should be at an angle pointed towards the well. The tip should be partially against the back plate of the gel cassette but the tip opening should be over the sample well, as illustrated in the figure on page 9.

   Do not try to jam the pipet tip in between the plates of the gel cassette.

4. Eject all the sample by steadily pressing down on the plunger of the automatic pipet.

   Do not release the plunger before all the sample is ejected. Premature release of the plunger will cause buffer to mix with sample in the micropipet tip. Release the pipet plunger after the sample has been delivered and the pipet tip is out of the buffer.

5. Before loading protein samples for the actual experiment, the practice gel loading solution must be removed from the sample wells.

   Do this by filling a transfer pipet with buffer and squirting a stream into the sample wells. This will displace the practice gel loading solution, which will be diluted into the buffer and will not interfere with the experiment.

EDVOTEK® Cat. #638, Fine Tip Micropipet Tips are recommended for loading samples into polyacrylamide gels. A regular microtip may damage the cassette and result in the loss of protein samples.

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Electrophoresis of Proteins (OPTIONAL)

READ ME!

EDVOTEK® Cat. #638, Fine Tip Micropipet Tips are recommended for loading samples into polyacrylamide gels. A regular micro-tip may damage the cassette and result in the loss of protein samples.

LOADING PROTEIN SAMPLES

Change pipet tips between loading each sample. Make sure the wells are cleared of all practice loading solution. Gently squirt electrophoresis buffer into the wells with a transfer pipet.

Two student groups can share a gel. Some of the samples contain denaturing solution which contains SDS and 2-mercaptoethanol. The samples should be loaded in the following manner:

First Student Group

Lane 1	20µl of Standard Protein Markers	(boiled for 5 minutes)
Lane 2	20µl of gfp native	(not boiled)
Lane 3	20µl of gfp denatured	(boiled for 5 minutes)
Lane 4	20µl of bfp native	(not boiled)
Lane 5	20µl of bfp denatured	(boiled for 5 minutes)

Second Student Group

Lane 6	20µl of Standard Protein Markers	(boiled for 5 minutes)
Lane 7	20µl of gfp native	(not boiled)
Lane 8	20µl of gfp denatured	(boiled for 5 minutes)
Lane 9	20µl of bfp native	(not boiled)
Lane 10	20µl of bfp denatured	(boiled for 5 minutes)

NOTE:

Shine the long wave U.V. light on the gel while the native proteins are separating.

RUNNING THE GEL

1. After the samples are loaded, carefully snap the cover all the way down onto the electrode terminals. The black plug in the cover should be on the terminal with the black dot.
2. Insert the plug of the black wire into the black input of the power supply (negative input). Insert the plug of the red wire into the red input of the power supply (positive input).
3. Set the power supply at the required voltage and run the electrophoresis for the length of time as determined by your instructor. When the current is flowing, you should see bubbles forming on the electrodes. The sudsing is due to the SDS in the buffer.
4. After the electrophoresis is finished, turn off power, unplug the unit, disconnect the leads and remove the cover.

Time and Voltage

Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min

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Staining the Gel

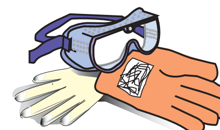
STAINING WITH PROTEIN INSTASTAIN® IN ONE EASY STEP

EDVOTEK features a state-of-the-art, proprietary stain for DNA or Protein gels called InstaStain®. Protein Polyacrylamide gels can be stained with Protein InstaStain® cards in one easy step. Staining is rapid, sensitive and Polyacrylamide gels are ready for visualization in 1-3 hours.

InstaStain® Blue and InstaStain® Ethidium Bromide are also available from EDVOTEK for staining of DNA gels.

1. After electrophoresis, turn off the power and remove the gel cassette from the gel electrophoresis apparatus.
2. To remove the gel from the cassette, lay the cassette down and carefully remove the front plate by placing a coin or a spatula in the slot at the top edge, near the sample wells, and twist to separate the two plates of the cassette.
3. Gently lift the front plate away from the larger back plate. In most cases, the gel will stay on the back plate. If the gel partially sticks to the front plate, let it fall onto the back plate.
4. Pour approximately 100 ml of fixative solution in a small tray.
5. Transfer the back plate of the cassette (with the gel) into the tray containing the fixative solution. Wet gloved fingers with fixative solution and gently nudge the gel off the back plate and remove the plate, leaving the gel submerged in the fixative solution.
6. Gently float a sheet of Protein InstaStain® card with the stain side (blue) facing in the liquid. Remove the Protein InstaStain® card after 30 minutes.
7. Cover the staining tray with saran wrap to prevent evaporation.
8. Gently agitate on a rocking platform for 1-3 hours or overnight.
9. After staining, Protein bands will appear medium to dark blue against a light background* and will be ready for excellent photographic results.

* *Destaining is usually not required but can be carried out if the gel background is too dark. Gels can be destained in several changes of fresh destaining solution until the appearance and contrast of the protein bands against the background improves.*



Wear gloves and safety goggles

NOTE:

Polyacrylamide gels are very thin and fragile. Use care in handling to avoid tearing the gel.

Fixative and Destaining Solution for each gel (100ml)

50 ml	Methanol
10 ml	Glacial Acetic Acid
40 ml	Distilled Water

STORING THE GEL:

Once satisfactory result is achieved, the gel can be stored in distilled or deionized water.

For permanent storage, the gel can be dried between two sheets of cellophane (saran wrap) stretched in an embroidery hoop. Air-dry the gel for several days until the gel is paper thin. Cut the "extra" saran wrap surrounding the dried gel. Place the dried gel overnight between two heavy books to avoid curling. Tape it into a laboratory book.

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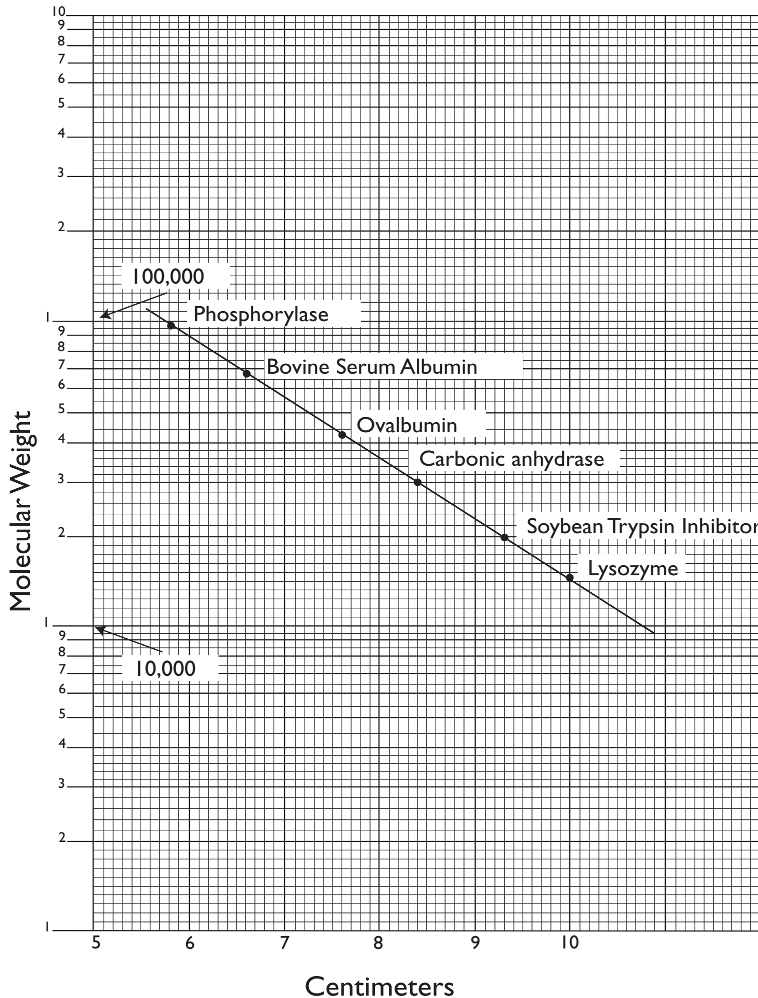
Determination of Molecular Weights

If measurements are taken directly from the gel, skip steps 1 and 2.

1. Take a transparent sheet, such as cellulose acetate (commonly used with overhead projectors) and lay it over the wrapped gel.

2. With a felt-tip pen, carefully trace the outlines of the sample wells. Then trace over all the protein bands on the gel.



In the example shown above, the standard molecular weights are:

94,000	30,000
67,000	20,000
38,000	14,000

3. Measure the migration distance, in centimeters (to the nearest millimeter) of every major band in the gel. (Ignore the faint bands, refer to Idealized Schematic.) All measurements should be from the bottom of the sample well to the bottom of the protein band.

4. Using semilog graph paper, plot the migration distance or Rf of each standard protein on the non-logarithmic x-axis versus its molecular weight on the logarithmic y-axis. Choose your scales so that the data points are well spread out. Assume the second cycle on the y-axis represents 10,000 to 100,000 (see example at left).

5. Draw the best average straight line through all the points. This line should roughly have an equal number of points scattered on each side of the line. As an example, refer to the figure at left. This method is a linear approximation.

6. Using your standard graph, determine the molecular weight of the three unknown proteins. This can be done by finding the Rf (or migration distance) of the unknown band on the x-axis and drawing a straight vertical until the standard line is intersected. A straight line is then made from the intersection across to the y-axis where the approximate molecular weight can be determined.

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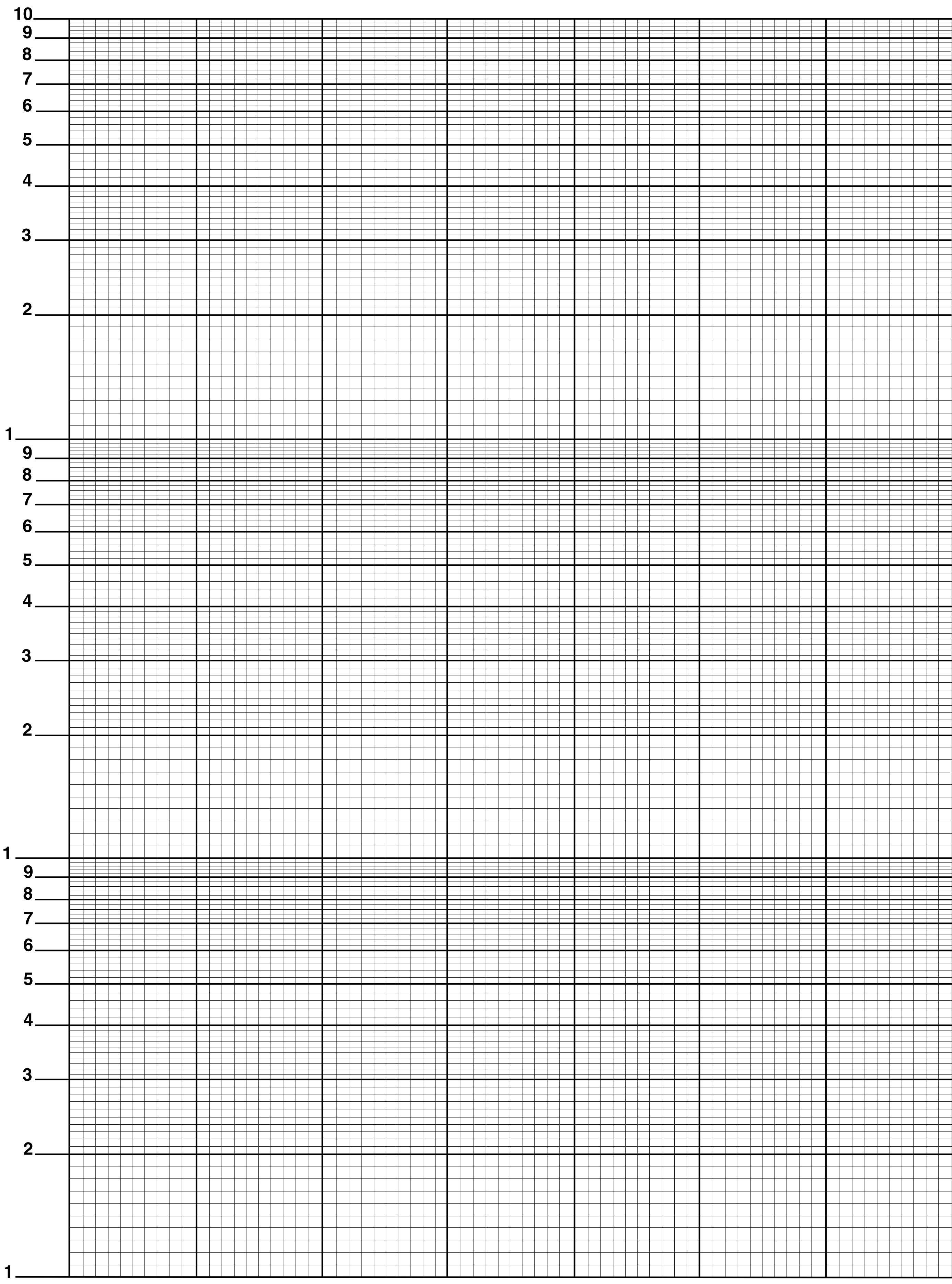
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Study Questions

1. What is the anticipated difference in apparent molecular weight between pFluoro-Green™ (gfp) and pFluoroBlue™ (bfp) as detected by denatured SDS- polyacrylamide gel analysis?
2. Why is the molecular sieving matrix swelled prior to packing the column?
3. What is the basis of molecular sieve chromatography?
4. Can molecular sieve chromatography columns be used to separate DNA fragments?

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Instructor's Guide

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HOW THIS EXPERIMENT IS ORGANIZED

This experiment module contains biologicals and reagents for six (6) groups sharing three (3) polyacrylamide gels (2 groups per gel). Enough buffer is included for three (3) vertical electrophoresis units (Model MV-10 or equivalent). Additional electrophoresis buffer is required for more than three units.

Note: Polyacrylamide gels are not included. You may choose to purchase precast gels (Cat. #s 651 or 652).

A variety of factors, such as class size, length of laboratory sessions, and availability of equipment, will influence the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

To facilitate implementation, this experiment can be divided into general experimental activities with logical stopping points. These optional stopping points can occur after each series of procedures.

Your personal preference and schedule will determine how and when the gels will be prepared for use in this experiment.

Technical Service Department

Mon - Fri
9:00 am to 6:00 pm ET

FAX: 202.370.1501
web: www.edvotek.com
email: info@edvotek.com

Please have the following information ready:

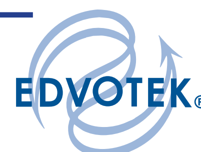
- **Experiment number and title**
- **Kit lot number on box or tube**
- **Literature version number (in lower right corner)**
- **Approximate purchase date**

APPROXIMATE TIME REQUIREMENTS FOR PRE-LAB AND EXPERIMENTAL PROCEDURES

1. Pre-lab preparations will require approximately 20 minutes on the day of the lab.
2. Students will require approximately 15 minutes to heat samples and load the gel. Practice gel loading may require an additional 15 minutes if performed the same day of the lab.
3. Electrophoresis will require approximately 1 to 1.5 hours, depending on the voltage.

PRACTICE GEL LOADING

This experiment kit contains practice gel loading solution. If your students are unfamiliar with vertical gel electrophoresis, it is suggested that they practice loading the sample wells before performing the actual experiment.



PreLab Preparations

COLUMN ELUTION BUFFER

Dilute the 10x Column Elution Buffer (C) by mixing 30ml of 10x Column Elution Buffer (C) with 270ml of Distilled water. Label this buffer "1x Column Elution Buffer".

PREPARATION OF THE SLURRY

1. Hydrate the Dry Matrix (E) in 40ml of 1x Column Elution buffer (diluted C).
2. Gently stir occasionally for a minimum of 30 to 60 minutes.
3. Aliquot 6ml for each of the six groups.

CELL EXTRACT CONTAINING GFP OR BFP FLUORESCENT PROTEIN

1. Thaw the frozen extracts (A and B) at room temperature and immediately place them on ice.
2. Label 6 tubes "gfp extract". Aliquot 220µl of the extract into the tubes. Place immediately back on ice.
3. Label an additional 6 tubes "bfp extract". Aliquot 220µl of the extract into the tubes. Place immediately back on ice.

For Partial Purification and Sample Preparation, each group requires:

1	Chromatography column
1	Ring stand with clamp
8	Microtest tubes
6ml	Slurry (hydrated E)
40ml	1x Column Elution Buffer (diluted C)
220µl	"gfp extract" (A)
220µl	"bfp extract" (B)
75µl	Protein Denaturing Solution (F)
75µl	50% Glycerol Solution (G)
	Automatic micropipet and tips
	5ml pipets and pump

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Pre-Lab Preparations

PREPARING ELECTROPHORESIS BUFFER

Prepare the electrophoresis buffer by adding and mixing 1 part Tris-Glycine-SDS 10x buffer concentrate to 9 parts distilled water.

The approximate volume of 1x electrophoresis buffer required for EDVOTEK® Protein Vertical Electrophoresis units are listed in the table below. The buffer should just cover the back plate of the gel cassette.

Tris-Glycine-SDS Electrophoresis (Chamber) Buffer

EDVOTEK Model #	Concentrated Buffer (10x)	+	Distilled Water	=	Total Volume
MV10	58 ml		522 ml		580 ml
MV20	95 ml		855 ml		950 ml

ELECTROPHORESIS TIME AND VOLTAGE

Your time requirements will dictate the voltage and the length of time for your samples to separate by electrophoresis. Approximate recommended times are listed in the table at right.

Time and Voltage

Volts	Recommended Time	
	Minimum	Optimal
125	60 min	75 min

RECONSTITUTION OF LYOPHILIZED PROTEIN MOLECULAR WEIGHT STANDARDS

Once rehydrated, the tube of Protein Molecular Weight Standards (D) contains enough material for loading 6 wells. The tube can be boiled in conjunction with the denatured GFP extracts.

1. Add 130 µl of distilled or deionized water to the tube of Potein Molecular Weight Standards (D) and allow the material to hydrate for several minutes. Vortex or mix vigorously.

2. Bring a beaker of water, covered with aluminum foil, to a boil. Remove from heat.

3. Make sure the sample tubes are tightly capped (in screw-cap tubes) and well-labeled. The bottom of the tubes should be pushed through the foil and immersed in boiling water for 5 minutes. The tubes should be kept suspended by the foil.

4. The markers can be aliquoted for each of the student groups, or students can share the rehydrated sample stock tube. **Have students load samples onto the polyacrylamide gel while the samples are still warm to avoid aggregation.** The volume of sample to load per well is 20 µl.

5. Store any unused portion of reconstituted sample at -20°C and repeat steps 2 and 3 when using samples at a later time.

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PreLab Preparations

PREPARING STAINING AND DESTAINING SOLUTIONS

The stock solution is used for staining and destaining with Protein InstaStain®

1. Solution for staining with Protein InstaStain®

- Prepare a stock solution of Methanol and Glacial Acetic Acid by combining 180 ml Methanol, 140 ml Distilled water, and 40 ml Glacial Acetic Acid.
- Staining of Protein Gel(s) is optional.

2. Destaining Solution

- Use the stock solution of Methanol, Glacial Acetic acid and distilled water (in Step 1) to destain the gel(s).

Electrophoresis Hints and Help

AVOIDING COMMON PITFALLS

- Use fresh electrophoresis buffer and stain, correctly diluted and prepared according to instructions.
- Before performing the experiment, practice sample delivery techniques to avoid diluting the sample with buffer during gel loading.

CARE AND MAINTENANCE OF THE ELECTROPHORESIS APPARATUS

To clean the apparatus chamber, rinse well with tap water. If your tap water is high in minerals, give the items a final rinse with distilled water. Let air dry. Do not use detergents of any kind, or expose the apparatus to alcohols. Avoid touching the fragile platinum electrodes.

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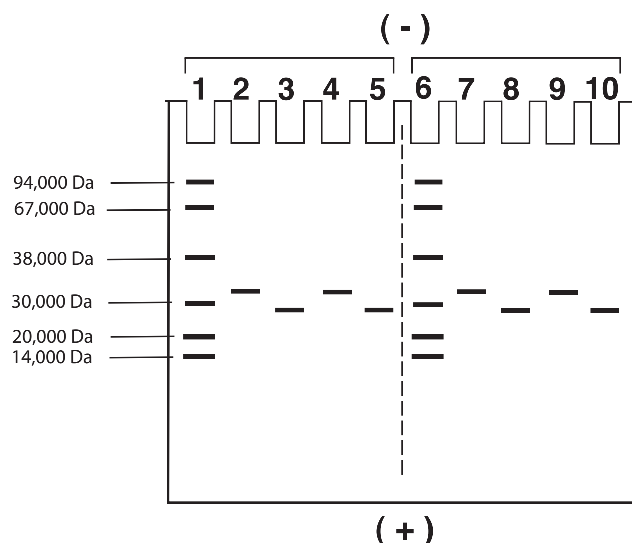
Idealized Schematic of Results



When proteins samples are boiled for 5 minutes in the presence of SDS and 2-mercaptoethanol, proteins lose their tertiary structure and are denatured. In the absence of boiling, complete denaturation is not achieved and local native structures can be maintained. The fluorescence observed in lanes 2, 4, 7 and 9, where samples were not boiled, could be due to core native structures that are responsible for the green and blue fluorescence of the gfp and bfp proteins respectively.

The size of the protein is about 40,000 for both bfp and gfp.

First Student Group

Lane 1	20µl of Standard Protein Markers	(boiled for 5 min.)
Lane 2	20µl of gfp native	(not boiled)
Lane 3	20µl of gfp denatured	(boiled for 5 min.)
Lane 4	20µl of bfp native	(not boiled)
Lane 5	20µl of bfp denatured	(boiled for 5 min.)

Second Student Group

Lane 6	20µl of Standard Protein Markers	(boiled for 5 min.)
Lane 7	20µl of gfp native	(not boiled)
Lane 8	20µl of gfp denatured	(boiled for 5 min.)
Lane 9	20µl of bfp native	(not boiled)
Lane 10	20µl of bfp denatured	(boiled for 5 min.)

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Please refer to the kit insert for the Answers to Study Questions

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Protein InstaStain

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW, Washington DC 20001

Emergency Telephone Number: 202-370-1500

Telephone Number for information: 202-370-1500

Date Prepared: 10-10-11

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Methanol (Methyl Alcohol) CH3OH	200ppm	200ppm	No data	90%-100%

Section III - Physical/Chemical Characteristics

Boiling Point	65°C	Specific Gravity (H_2O = 1)	.79
Vapor Pressure (mm Hg.)	96mmHg	Melting Point	N/A
Vapor Density (AIR = 1)	1.11	Evaporation Rate (Butyl Acetate = 1)	4.6

Solubility in Water: Complete (100%)

Appearance and Odor: chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): (closed cup) 12°C

Flammable Limits: LEL 6.0% UEL 36%

Extinguishing Media: Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective)

Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. Move containers from firearea

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources. Close containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability: Unstable [ ] Stable [X] — Conditions to Avoid: None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, Sulfur oxides

Hazardous Polymerization: May Occur [ ] Will Not Occur [X] — Conditions to Avoid: None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Irritating to eyes, skin, mucous membranes and upper respiratory tract. Chronic exposure may cause lung damage or pulmonary sensitization

Carcinogenicity: NTP? No data IARC Monographs? No data OSHA Regulation? No data

Signs and Symptoms of Exposure: Respiratory tract: burning sensation. Coughing, wheezing, laryngitis, shortness of breath, headache

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Flush skin/eyes w/ large amounts of water. If inhaled, remove to fresh air. Ingestion: give large amounts of water or milk. Do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up w/ absorptive material and burn in chemical incinerato equipped w/ an afterburner and scrubber.

Waste Disposal Method: Observe all federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Wear protective gear. Avoid contact/inhalation.

Other Precautions: Strong sensitizer

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation: Local Exhaust: No; Special: Chem fume hood; Mechanical (General): No; Other: None

Protective Gloves: Rubber — Eye Protection: Splash-proof goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid prolonged or repeated exposure

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Practice Gel Loading Solution

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW, Washington DC 20001

Emergency Telephone Number: 202-370-1500

Telephone Number for information: 202-370-1500

Date Prepared: 10-10-11

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)] — OSHA PEL — ACGIH TLV — Other Limits Recommended — % (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): No data

Flammable Limits: LEL No data UEL No data

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability: Unstable [ ] Stable [X] — Conditions to Avoid: None

Incompatibility: None

Hazardous Decomposition or Byproducts: Sulfur oxides, and bromides

Hazardous Polymerization: May Occur [ ] Will Not Occur [X] — Conditions to Avoid: None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: May cause irritation. No data available for other routes.

Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type)

Ventilation: Local Exhaust: Yes; Special: None; Mechanical (General): Yes; Other: None

Protective Gloves: Yes — Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Tris-Glycine SDS Running Buffer (10X)

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th Street NW, Washington DC 20001

Emergency Telephone Number: 202-370-1500

Telephone Number for information: 202-370-1500

Date Prepared: 10-10-11

Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Tris CAS# 77-86-1				3%
Glycine CAS# 56-40-6				14%
Sodium dodecyl sulfate CAS# 151-21-3				1%

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Clear, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): No data

Flammable Limits: LEL No data UEL No data

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder or appropriate foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing

Unusual Fire and Explosion Hazards: May emit toxic fumes

Section V - Reactivity Data

Stability: Unstable [ ] Stable [X] — Conditions to Avoid: Strong oxidizing agents, strong acids

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, sulfur oxides, sodium oxides

Hazardous Polymerization: May Occur [ ] Will Not Occur [X] — Conditions to Avoid

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): May cause irritation to eyes, skin, and mucous membranes.

Carcinogenicity: No data NTP? No data IARC Monographs? No data OSHA Regulation? No data

Signs and Symptoms of Exposure: Irritation

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: Skin/eye contact: flush w/ water. Inhalation: remove to fresh air. Ingestion: Seek medical attention

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear protective clothing. Avoid contact. Mop up with absorbant material and dispose of properly.

Waste Disposal Method: Follow all state, federal, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid contact, keep away from heat.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type)

Ventilation: Local Exhaust: No; Special: None; Mechanical (General): Yes; Other: None

Protective Gloves: Chem resistant — Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Lab coat, coveralls

Work/Hygienic Practices: Prevent contact

Material Safety Data Sheets

Full-size (8.5 x 11”) pdf copy of MSDS is available at www. edvotek.com or by request.

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Protein Denaturing Solution

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th St NW, Washington DC 20001

Emergency Telephone Number: 202-370-1500

Telephone Number for information: 202-370-1500

Date Prepared: 12-02-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Lauryl Sulfate, Sodium	No data	No data	No data	No data
C12H26O4S				
CAS # 151-21-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Blue liquid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): No data

Flammable Limits: LEL: No data; UEL: No data

Extinguishing Media: Water spray, Carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes.

Unusual Fire and Explosion Hazards: May emit toxic fumes.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon Monoxide, Carbon Dioxide, Sulphur oxides

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes; Skin? Yes; Ingestion? Yes

Health Hazards (Acute and Chronic): Irritating to eyes, skin, mucous membranes and upper respiratory tract. Chronic exposure may cause lung damage or pulmonary sensitization resulting in hyperactive airway dysfunction.

Carcinogenicity: Not Known; NTP? No data; IARC Monographs? No data; OSHA Regulation? No data

Signs and Symptoms of Exposure: Respiratory tract: Burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If inhaled, remove to fresh air. Ingestion: Large amounts of water or milk. Do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Wear SCBA apparatus, rubber boots and rubber gloves. Mop up w/absorptive material and burn in chemical incinerator equipped w/ afterburner & scrubber.

Waste Disposal Method: Observe all federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Wear protective gear. Avoid contact/inhalation.

Other Precautions: Strong sensitizer

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation: Local Exhaust: No; Special: Chemical fume hood; Mechanical (General): No; Other: None

Protective Gloves: Rubber; Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid prolonged or repeated exposure

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Dry Matrix

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 1121 5th St NW, Washington DC 20001

Emergency Telephone Number: 202-370-1500

Telephone Number for information: 202-370-1500

Date Prepared: 12-02-11

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Data not available				
CAS# 9050-94-6				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: No data

Appearance and Odor: White powder

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): No data

Flammable Limits: LEL: No data; UEL: No data

Extinguishing Media: Water spray, Carbon dioxide, dry chemical powder, foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes.

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes; Skin? Yes; Ingestion? Yes

Health Hazards (Acute and Chronic): Irritating

Carcinogenicity: NTP? Yes; IARC Monographs? Yes; OSHA Regulation? Yes

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Skin/eye contact: Flush eyes with copious amounts of water. Inhalation: Remove to fresh air. Ingestion: Wash out mouth with water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up, place in a bag and hold for waste disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full face mask

Ventilation: Local Exhaust: Yes; Special: ; Mechanical (General): Yes; Other: Yes

Protective Gloves: Yes; Eye Protection: Yes

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: Do not ingest. Avoid contact with skin/eyes.