Protein power!

Student's guide

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Every part of this burger contains proteins. There are proteins in the meat, cheese, bread, lettuce — and even in the seeds on top. Every cell and tissue in your body contains protein too. It's about one-fifth of your total body weight. Proteins are a major part of the structure of your hair, nails and skin. Proteins are also what makes you 'tick'. They work as enzymes to speed up chemical reactions and as hormones and neuro-transmitters, carrying vital messages throughout your body.

Specialised proteins called antibodies help to protect from infection by 'recognising' the proteins of invading microbes. Many more proteins are constantly repairing body tissues to keep you healthy.

What are proteins made from?

Proteins are made of long chains of amino acids. There are 20 different amino acids. Rather like the alphabet, these amino acid 'letters' can be arranged to create thousands of different 'words' — that is, different proteins. The sequence of amino acids in the various chains causes them to fold and coil in different ways. It's the 3-D shapes of these chains that allow proteins to perform such a wide range of jobs.

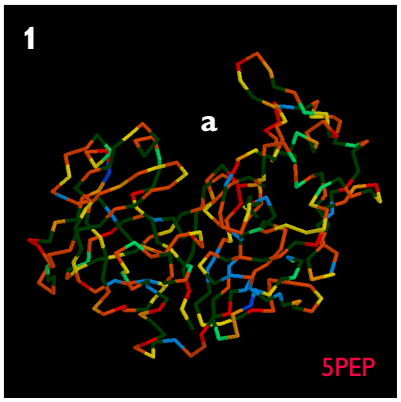
Where do proteins come from?

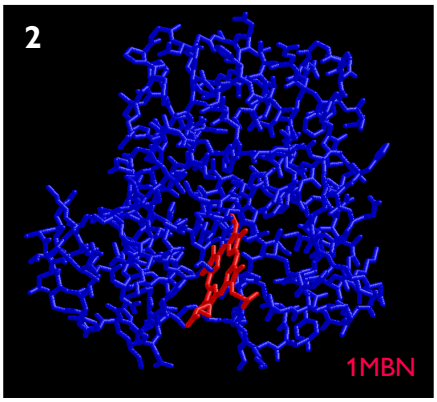
Plants make their own proteins using nutrients from the soil and carbon dioxide from the air. Animals digest proteins they eat, breaking them down into amino acids. These amino acids are absorbed and reassembled to make the animals' own proteins.

In all living things, the instructions for making proteins are encoded in DNA. We now know a lot about our own DNA and that of other organisms. Increasingly, however, the focus of research is shifting from the instruction manual (the DNA) to the product of those instructions — the proteins.

For instance, powerful computers are being used to try to predict the shape of proteins from their sequences of amino acids. This will help us to understand how proteins work and perhaps more importantly, why they sometimes don't work properly when the shape of the protein is changed.

Computer-generated models of proteins *(not to scale).*

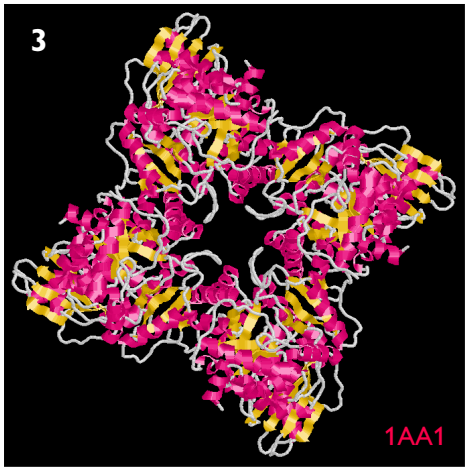




Data for these computer models came from the Protein Data Bank (www.rcsb.org/pdb).

The codes in red are those you should type in if you want to look the proteins up.

If you download the computer programme RASMOL, you can examine these proteins in glorious 3-D. Details are given on the back page.

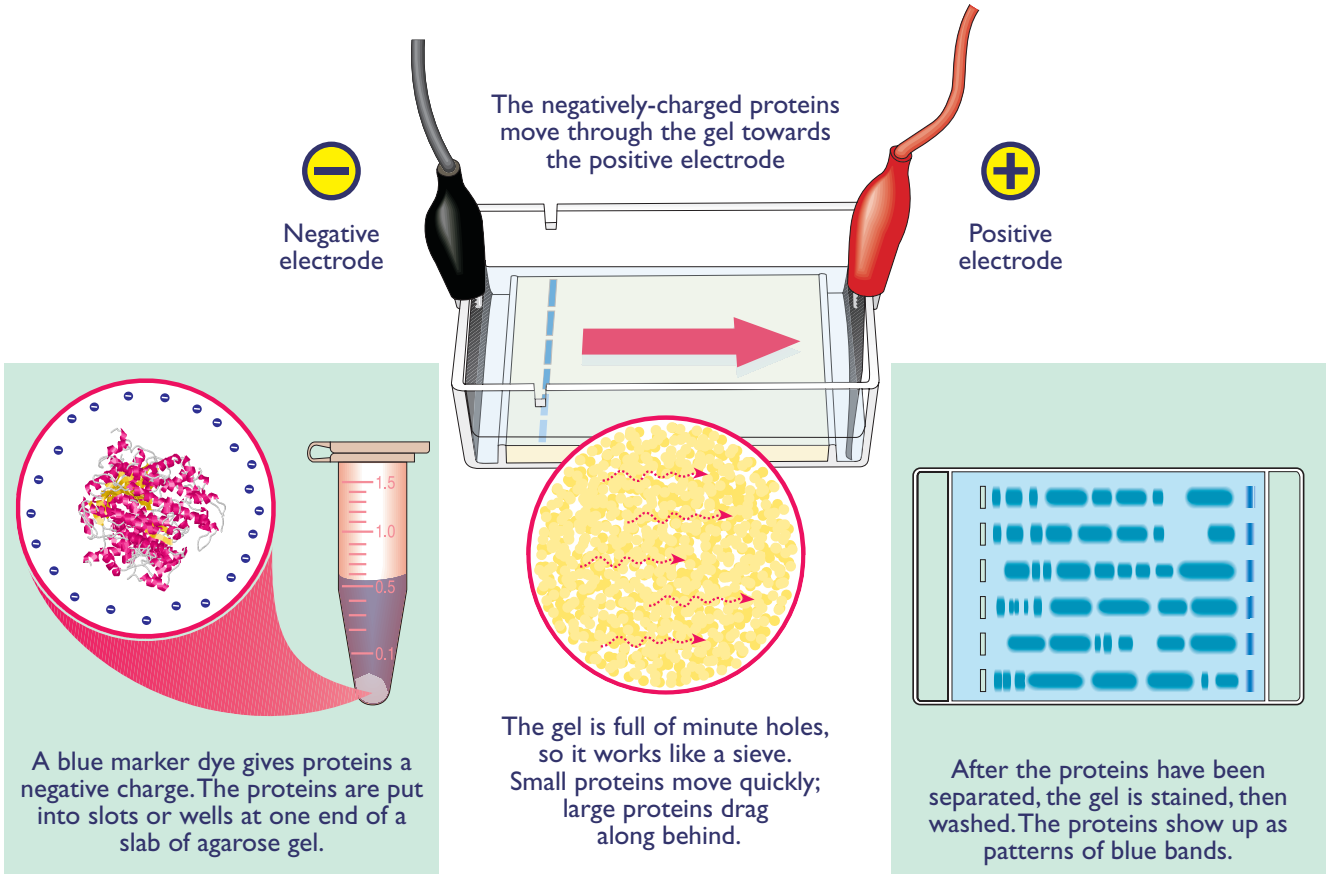


1. Pepsin. *The first enzyme to be discovered. In the stomach, pepsin helps to digest other proteins. Each of the different colours in the chain is a different amino acid. The active site of the enzyme (a) is where the proteins being digested bind to it.*

2. Myoglobin*. An oxygen-storage protein found in muscles. Myoglobin was the first protein structure to be worked out, by John Kendrew and his colleagues in 1960. In this picture, the amino acid part of the protein is shown in blue, with the oxygen-carrying part in red.*

3. Rubisco. *This is the commonest enzyme on Earth. The chloroplasts of green plants are stuffed with it. It takes carbon dioxide from the air and attaches it to another molecule — part of the process of making sugar during photosynthesis. For an enzyme, Rubisco is painfully slow, taking up just three molecules of carbon dioxide per second.*

Separating proteins



Electrophoresis (which means *'carrying with electricity'*) is used to separate proteins or protein fragments of different sizes. This is done by making the proteins move through a porous slab of jelly (gel). The gel is held in a small tank. At one end of the gel are slots into which the proteins are put. An electrical current makes the proteins move through the gel. After electrophoresis the gel is stained, then washed, to reveal the different proteins. Each separate band on the gel is made up of millions of protein molecules of roughly the same size.

You can use this method to compare the proteins that are found in different samples of fish, in seeds or even in biological washing powders or breakfast cereals.

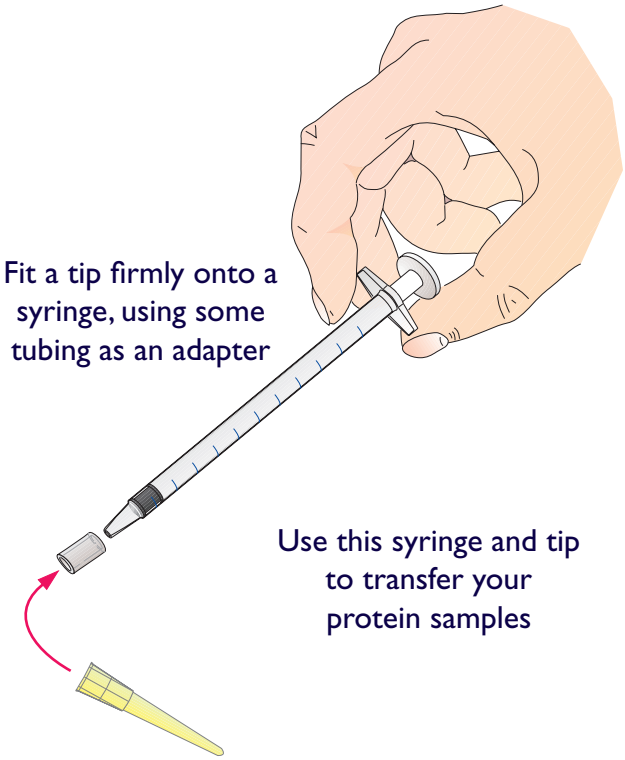
Ideas for investigations (the teacher's guide gives more suggestions)

- Investigate differences in the muscle proteins from different types of fish and shellfish. Can you explain any differences you find in terms of evolutionary relationships?
- Are 'crabsticks' made of crab? If not, what are they made of?
- Fish fingers vary in price and quality. Can you find differences in the fish used for different quality fish fingers?
- How do the proteins found in cheeses alter as the cheese matures? Compare, for instance, mild, mature, and vintage cheddar cheeses.
- What differences are there between the proteins found in seeds from the grass family (*e.g.*, rice, wheat, barley) and those from the pea family (such as peas, beans and dal).
- Soak some seeds in water, then divide them up into parts (*e.g.,* root, cotyledons, embryo). Do the different parts contain different proteins?
- What difference does cooking make to proteins? Try raw, cooked and whisked egg white.
- Biological washing powders contain different enzymes (*e.g.*, amylases, proteases and lipases). These enzymes are found in little granules in the powder. Separate out the granules from different types of washing powder then use electrophoresis to see what enzymes they contain.
- How does the shape of a protein affect the way in which it moves on a protein gel? Use the *Protein Data Bank* to search for proteins and to discover their shapes (more details of the *Protein Data Bank* are given on the back page).

1 Measuring small volumes

In this work, you will need to measure small volumes of liquid. To do this you can use a syringe fitted with a disposable tip. A small piece of soft rubber tubing makes sure that the tip stays firmly on the syringe.

So that your different samples remain pure, you should use each tip only once, then throw it away. *Take care, however, not to throw the rubber tubing away!*

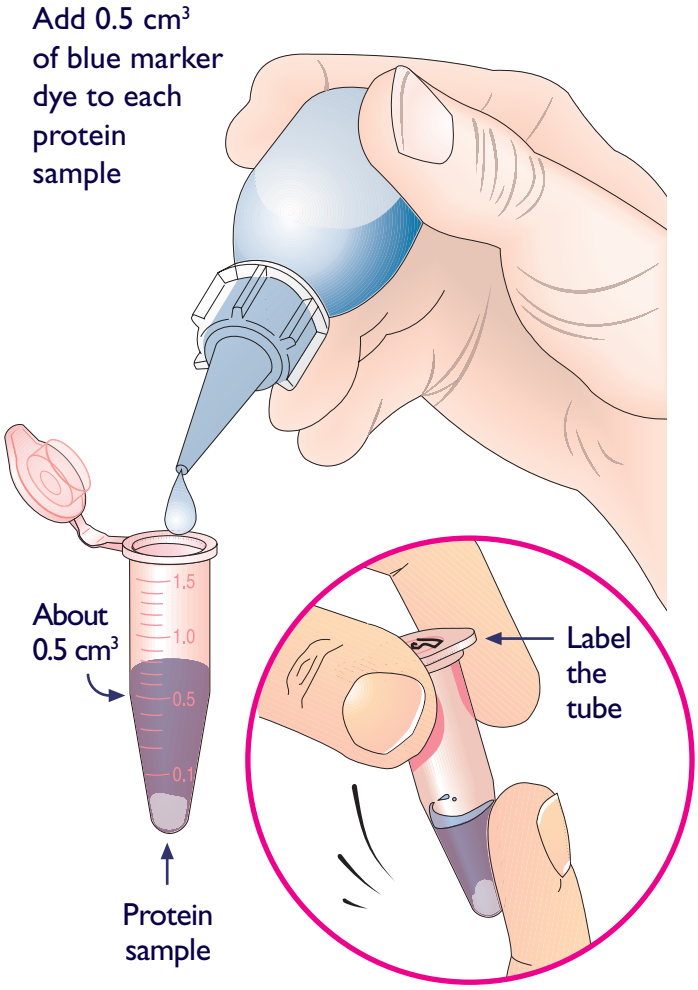


2 Preparing the protein samples

Most food samples (such as fish and cheese) can be used without any special preparation. If you use seeds, however, you will need to grind them into a fine powder first.

After you have prepared the food sample(s):

a. Place each of your protein samples in a clean tube. *It is very important to use small samples — an amount about half the size of a grain of rice is about right.*
b. Add 0.5 cm^3 of blue marker dye to each tube.
c. Close the tubes and label them using a waterproof pen. *Write near the rim and on the cap where the number is less likely to rub off.*
d. Flick each tube 15–20 times with your finger to mix its contents. *Some of the proteins from the sample will now dissolve in the marker dye.*
e. Leave the tubes to stand upright for about 5 minutes so that any large solid bits sink to the bottom of the tubes.



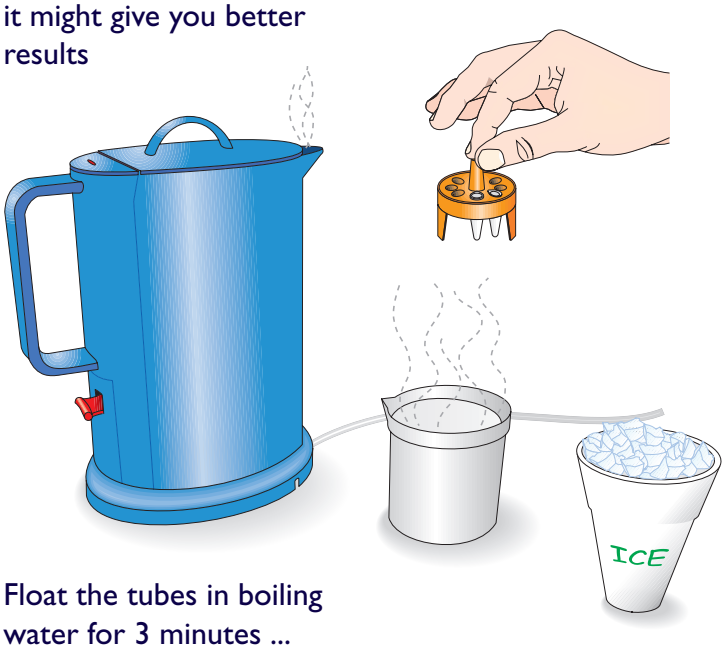
3 OPTIONAL STEP
Boiling the samples

You don't need to do this step, but it can help you to obtain clearer results. Why is this?

The heating changes the shape of the proteins so that they unwind and become long chains. All of the proteins end up the same shape, so they will therefore move through the gel according to their size rather than the way they are folded.

a. Check that the tubes are closed tightly and are clearly labelled so that the ink will not rub off .
b. Put the tubes into a floating holder, then place them in a boiling water bath or beaker of boiling water for about 3 minutes. *Take care not to scald yourself as you do this!*
c. Take the tubes out of the water and store them in crushed ice until you are ready for the next step.

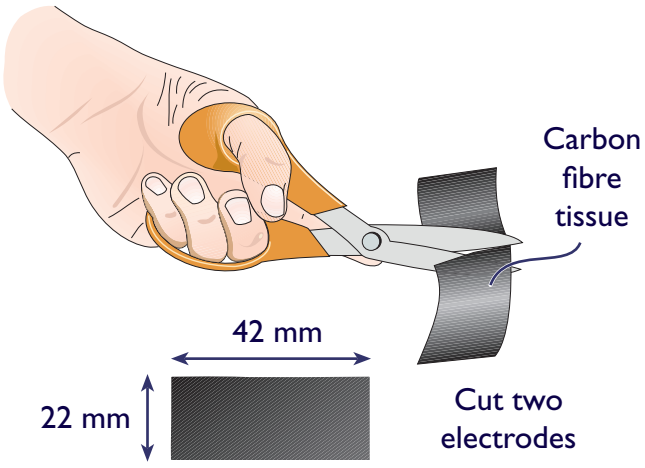
You don't need to carry out this step, but it might give you better results

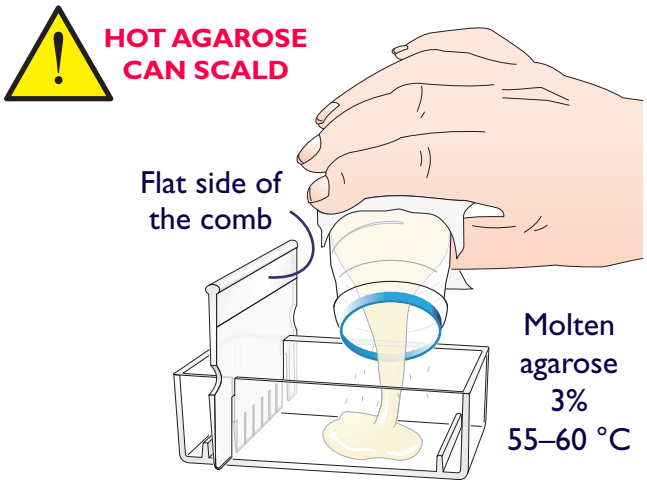


Float the tubes in boiling water for 3 minutes ...
... then store them on ice.

4 Preparing the gel

a. Cut two pieces of the carbon fibre tissue. These will be the electrodes at either end of the gel tank. Test that the electrodes fit neatly at the ends of the tank, then take them out and put them to one side.
b. Place a 6-toothed comb into the slot at one end of the gel tank, so that the flat side faces the way shown in the picture. Stand the tank on a *level* surface.
c. *Taking care not to scald yourself*, pour just over 10 cm^3 of molten gel into the tank so that it fills the centre and flows under and between the teeth of the comb. Try not to add too much molten gel — you need a flat gel, not one that curves upwards. If you spill gel into the areas at either end of the tank, just leave it to set — you can scoop it out later once it has hardened.
d. Leave the tank undisturbed for 15–20 minutes, until the gel hardens (the gel will turn slightly milky as it sets).





5 Loading the gel

a. Pour slightly more than 10 cm^3 of TB buffer solution into the gel tank. The liquid should cover the surface of the gel and flood into the areas at either end, making a layer over the gel about 2 mm deep. *The buffer will conduct electricity through the gel.*

b. *Very gently* ease the comb from the gel, taking care not to tear the wells.

c. Put the gel tank where it will not be disturbed while the gel is being 'run'.

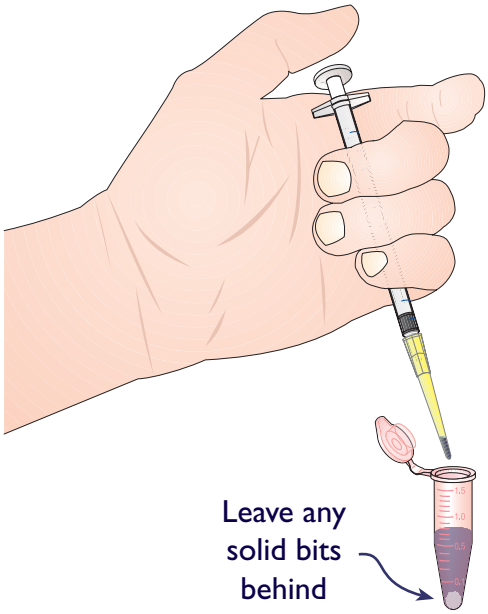
It will be easier to see what you are doing next if you place the gel tank on a dark surface, such as a piece of black paper.

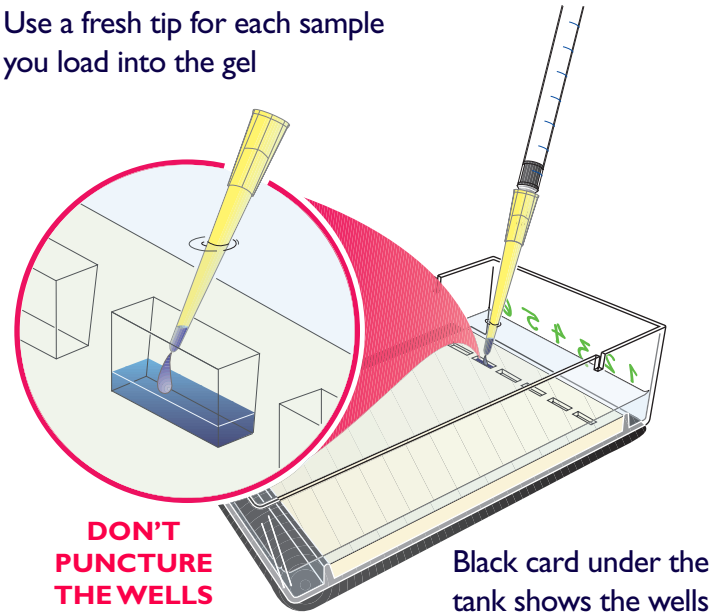
d. Make a note of which protein sample you will put into each well (write on the side of the gel tank with a marker pen if necessary).

e. Put a clean tip on the syringe. Draw up some of the liquid from the top of your protein sample. You don't need much; about 5 mm in the tip will be enough. *Make sure that you just take liquid from the top of the tube — no bits!*

f. Transfer your protein sample into one of the wells, holding the tip above the well but just in the buffer solution (see picture). *Take great care not to stab the bottom of the well with the tip.*

g. Repeat steps **e** and **f** with the other samples (perhaps from other people in your class).
IMPORTANT! Remember to use a new tip to transfer each sample.

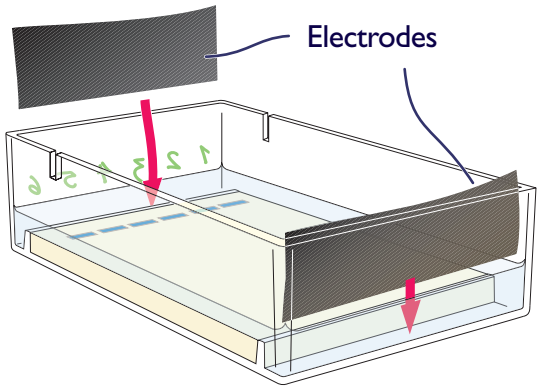




6 'Running' the gel

a. Fit one electrode at each end of the tank as shown in the picture. Join the electrodes to batteries using wires with crocodile clips. Sufficient batteries (in series) should be used to give a total voltage of no more than 36 volts. *Ensure that the positive terminal of the battery is connected to the electrode* ***furthest*** *from the wells.*

Serious or lethal electrical shock could result if the equipment were to be connected directly to a mains socket.

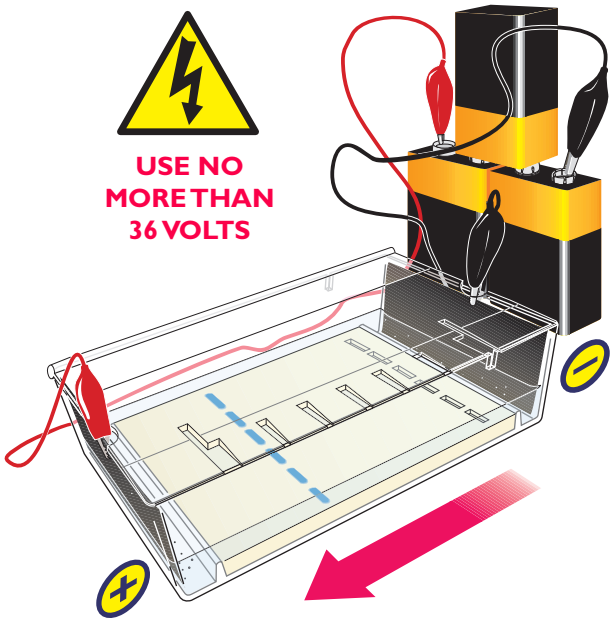


b. Ensure that contact is made between the buffer solution and the electrodes (add a little more TB buffer if necessary). *You should see bubbles coming from the negative electrode if a current is flowing.*

c. *Use the 6-toothed comb as a lid over the gel tank. This will stop the gel from drying out.* Leave the gel to 'run', undisturbed, for several hours. The large red arrow on the picture shows which way the proteins move.

d. Disconnect the batteries when the blue marker dye is near the far end of the gel. *If you leave the batteries connected, the proteins will run off the end of the gel!*

e. Rinse the crocodile clips in tap water and dry them thoroughly to prevent rusting.



Place the comb over the tank to reduce evaporation

7 Staining the proteins

a. Remove and throw away the electrodes. Pour away the buffer solution.

b. Leave the gel in the tank. Wash the gel with distilled or deionised water, then pour some water into the tank and leave the gel to soak for about 1 hour. Change the water at least once during this time. *It is important to remove as much of the buffer as possible if the proteins are to be properly stained in the next step.*

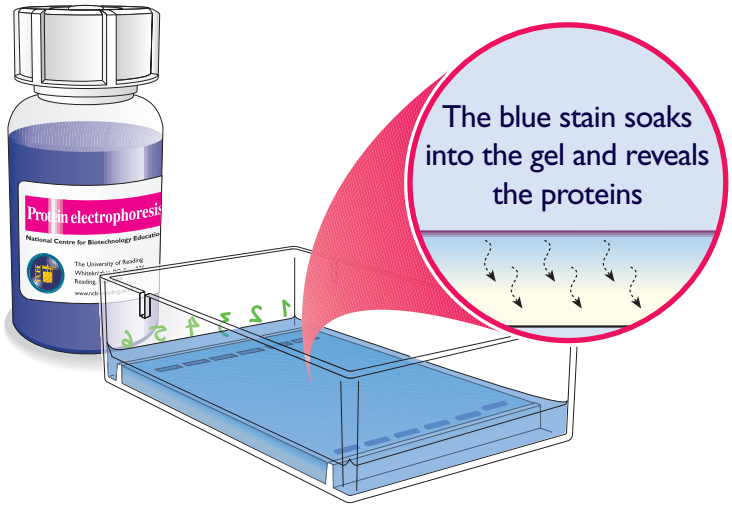
c. ***Shake the stain bottle well.*** Pour about 12 cm^3 of blue protein-staining solution onto the gel.

d. Cover the gel tank with the 6-toothed comb or put the tank in a plastic bag so that the liquid does not evaporate. Leave the tank overnight so that the stain soaks into the gel. *The blue stain will bind to the proteins.*

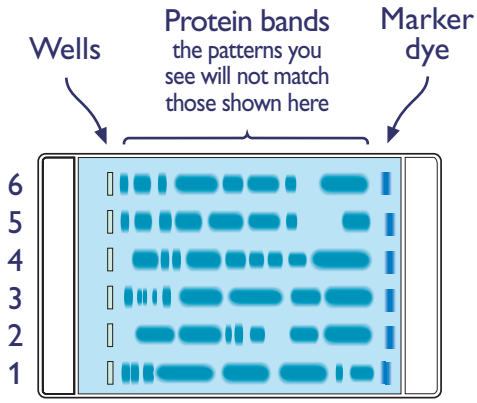
e. Pour away the blue stain then rinse the surface of the gel very carefully with distilled or deionised water.

f. At this point, both the proteins and the gel itself will be stained blue. You now need to remove the stain from the gel so that the protein bands can be seen clearly. Add some distilled or deionised water to the tank and leave it for 4–6 hours. Change the water after 2–3 hours.

g. Study the pattern of bands on the gel. The different bands are different proteins (or fragments of proteins). The best results will be seen 24–48 hours after destaining, or even later.



After staining and washing the gel:



Dean Madden

SAFETY GUIDELINES

Agarose gel

If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an *unsealed* container. If a microwave oven is not available, a boiling water bath or hotplate may be used instead. The gel must be swirled as it melts to prevent charring. The use of a Bunsen burner to melt agarose is *not* recommended.

WARNING! Hot, molten agarose can scald and it must be handled with care.

TB buffer (Tris-Borate)

When used as directed, this buffer presents no serious safety hazards. Splashes in the eye of on the skin should be washed away promptly with water. Spent buffer can be washed down the drain.

Electrode tissue

The carbon fibre electrode tissue may release small fibres, which can cause minor skin irritation if you handle the tissue a lot. Wear protective gloves if you find the tissue unpleasant to handle. The fibres released are too large to enter the lungs however, so it is not necessary to wear a face mask. In addition, the fibres are soluble in body fluids and are completely biodegradable.

Electrical supply

WARNING! The gel electrophoresis equipment was designed to be used at low voltages (no more than 36 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as the live electrical components are not isolated from the user.

WARNING! Serious or lethal electrical shock could result if the equipment were to be connected directly to a mains power supply.

Protein stain (Colloidal Coomassie Blue G 250)

The Colloidal Coomassie Blue is dissolved in water and is safe to handle. It presents no serious safety hazards, although care should be taken to prevent splashes on the skin or eyes *e.g., wear protective gloves and safety glasses.* Used stain can be diluted with water and washed down the drain.

Blue marker dye (Laemmli buffer)

When used as directed, Laemmli buffer presents no serious hazards. It contains a blue dye, a detergent to give proteins a negative charge and glycerol to make it heavy. Used buffer can be washed down the drain.

WEB SITES & MORE INFORMATION

The Biotechnology and Biological Sciences Research Council

The Biotechnology *and Biological Sciences Research Council* funds research and training in the non-medical life sciences. The BBSRC has an on-line exhibition on its Web site at:

www.bbsrc.ac.uk/life/index.html

Science and Plants for Schools

The SAPS Web site offers ideas for investigations, practical worksheets, details of the SAPS work-shop calendar, practical kits and sponsorship as well as access to a selection of plant science publications. There is also an enquiry service.

www.saps.org.uk

National Centre for Biotechnology Education

The NCBE, which is part of the *School of Food Biosciences* at The University of Reading, has more ideas for protein electrophoresis on its Web site, including short video clips of how to carry out the practical work:

www.ncbe.reading.ac.uk

The Protein Data Bank

The *Protein Data Bank* has information about thousands of proteins, put there by scientists from around the world. At the *Protein Data Bank* Web site you can see computer-generated images of proteins and download data for exploring their 3-D structures on your own computer (using *RASMOL*).

The *Protein Data Bank* has fascinating special features on proteins in its *'Molecule of the Month'* series. These include the muscle proteins, actin and myosin:

Myosin

www.rcsb.org/pdb/molecules/pdb18_1.html

Actin

www.rcsb.org/pdb/molecules/pdb19_1.html

RASMOL

The molecular modelling software *RASMOL* will help you to view protein structure data from the *Protein Data Bank*. *RASMOL* is available free-of-charge from:

www.umass.edu/microbio/rasmol

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