Protein electrophoresis

What is it?

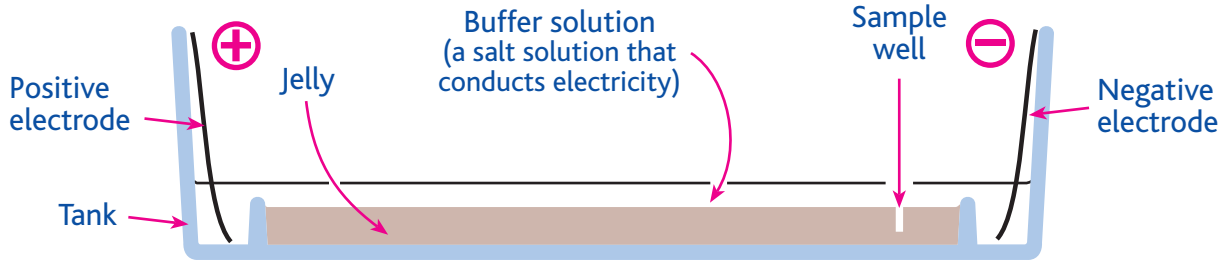
Electrophoresis ('carrying with electricity') is a technique used to separate large molecules such as **proteins**.

How does it work?

First, the proteins are given a **negative electrical charge**.

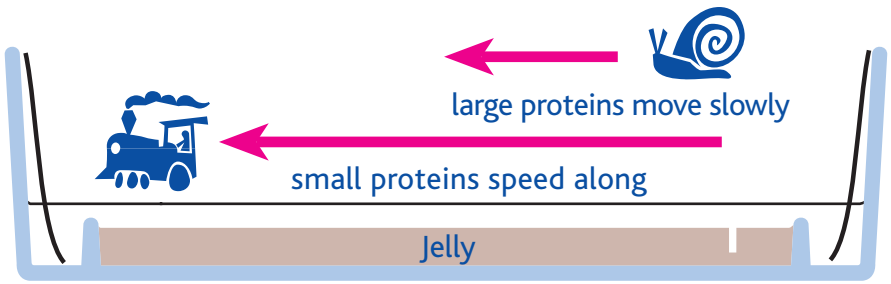
The protein samples are put into slots or 'wells' at one end of a slab of jelly. The samples are heavy, so they sink to the bottom of the wells.

A side view of the equipment:



When the power is switched on the molecules move out of the wells and through the jelly towards the positive electrode. A blue dye shows how far the proteins have gone.

Large proteins move slowly; small ones move more quickly. In this way they are separated by size. Usually the proteins must be stained so that they can be seen after separation.



Why is it important?

Electrophoresis is one of the most important techniques used by biologists today. You can use it to test what sort of proteins there are in the food you eat.

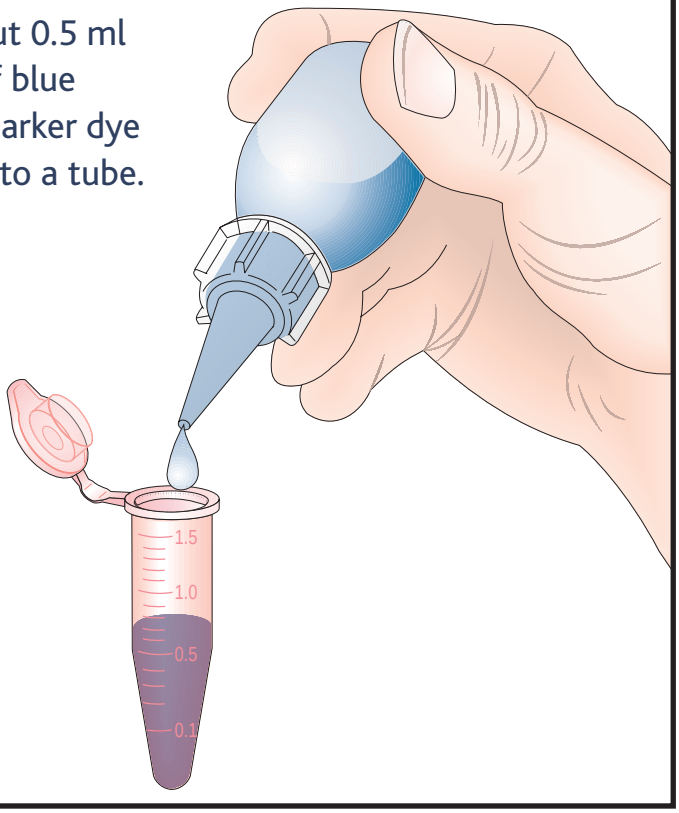
Proteins from fish

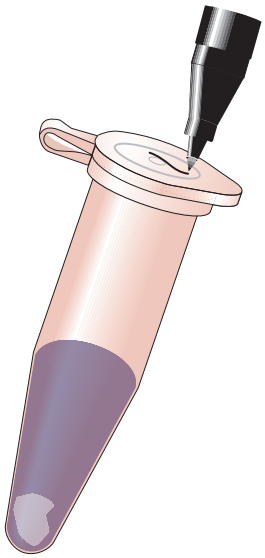
Here's a simple method of extracting proteins from fish. It also works for shellfish — things like crabs, mussels or prawns. You can use fresh or frozen fish, but fresh fish usually gives better results. You'll need to repeat this method for each type of fish you want to test.

Cut a tiny piece of fish about half the size of a pea.

DON'T CHOP YOUR FINGERS!

Put 0.5 ml of blue marker dye into a tube.

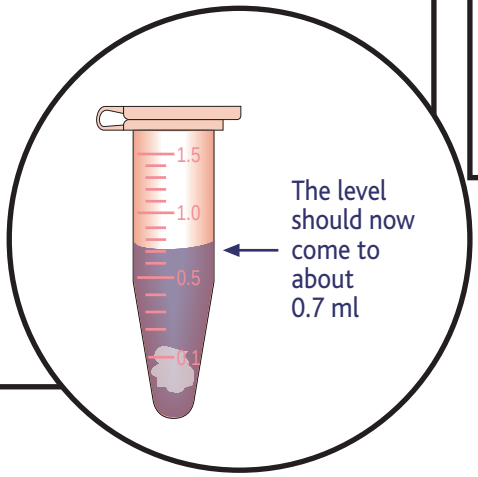




Add the fish to the tube and label it like this:

JS ← your initials

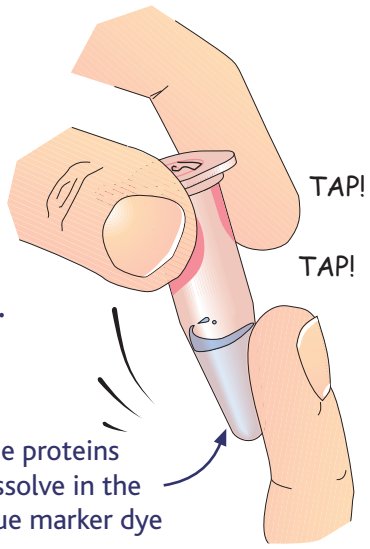
Cod ← type of fish



The level should now come to about 0.7 ml

Close the tube, tap it 15–20 times to mix the contents ...

... then let the tube stand for 5 minutes.



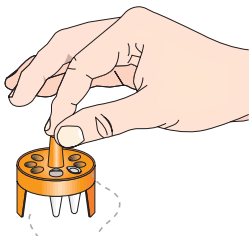
Now you've got the proteins, you can separate the different types by protein electrophoresis.

Protein electrophoresis

Electrophoresis ('carrying with electricity') is a way of separating different proteins.

Samples of the proteins are put in slots or 'wells' at one end of a slab of jelly. An electrical current is applied and the proteins move out of the wells and through the jelly. Large proteins move slowly through the jelly; smaller ones move more quickly. In this way the proteins are separated by size. Usually you need to stain the proteins after they have been separated to see them.

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

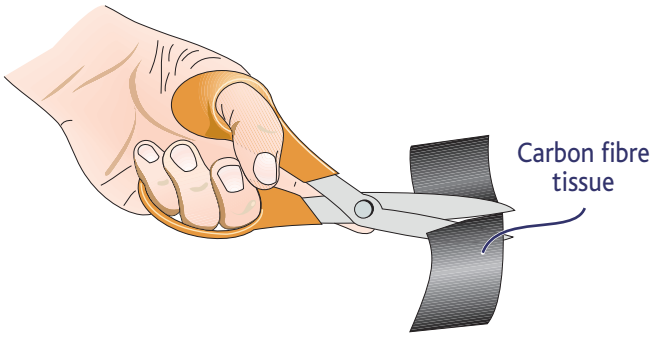


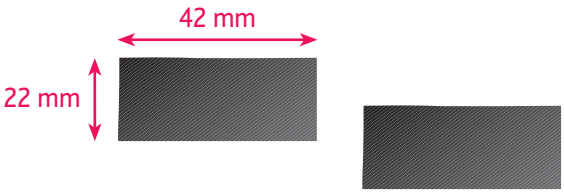
DON'T SCALD YOURSELF!

Float the tubes in boiling water for 3 minutes ...

... then store them on ice.

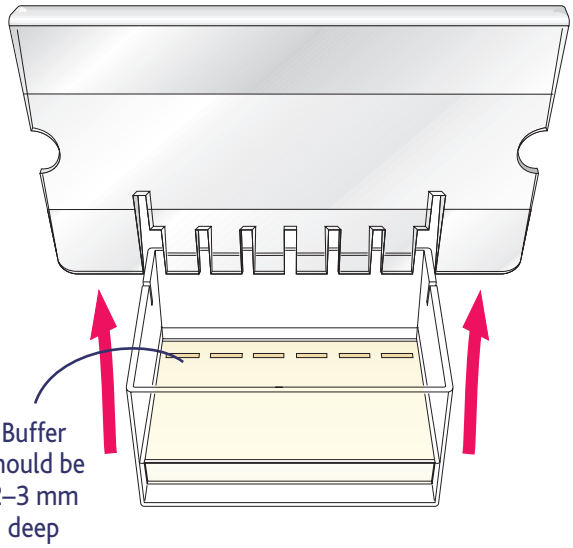
Cut 2 electrodes from carbon fibre tissue.





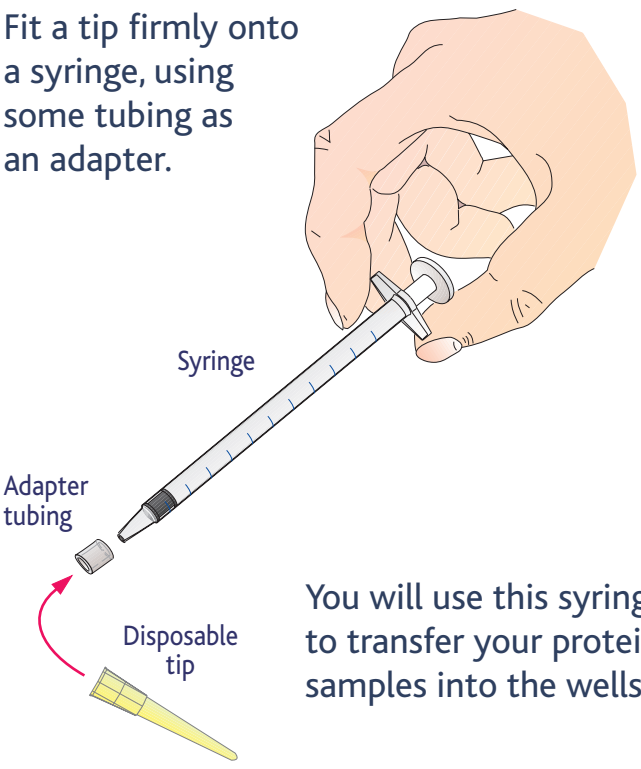
Pour buffer solution over the jelly ...

... then take the comb out gently ...

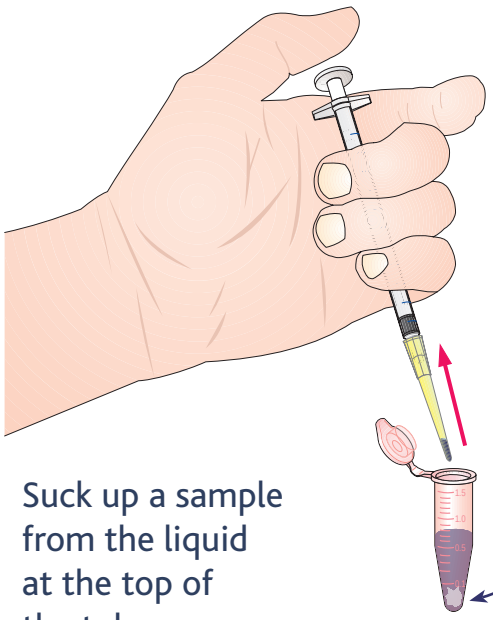


... leaving 6 'wells', ready for your samples.

Fit a tip firmly onto a syringe, using some tubing as an adapter.



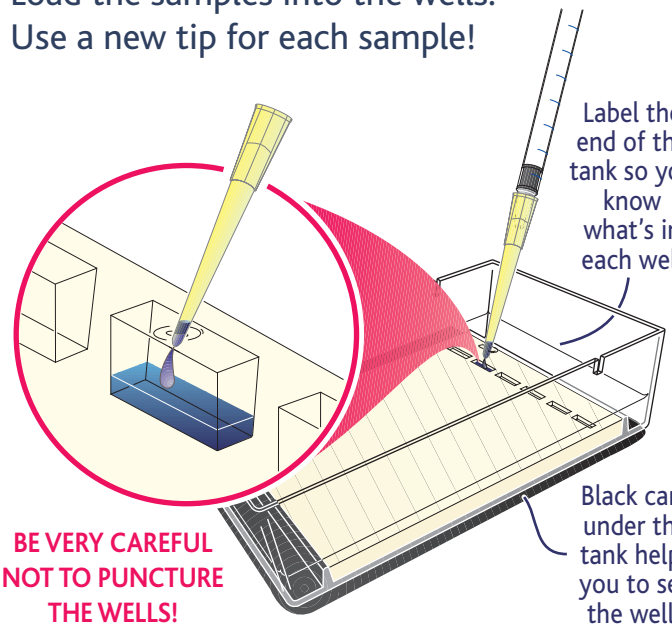
You will use this syringe to transfer your protein samples into the wells.



Suck up a sample from the liquid at the top of the tube.

Leave any solid bits behind

Load the samples into the wells.
Use a new tip for each sample!



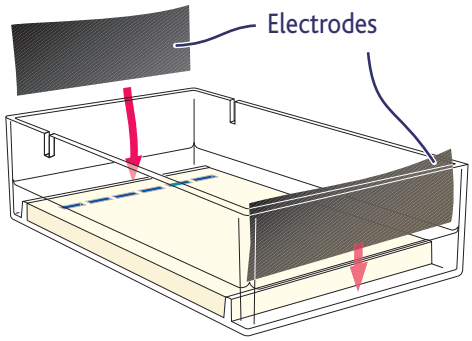
Label the end of the tank so you know what's in each well

BE VERY CAREFUL NOT TO PUNCTURE THE WELLS!

Black card under the tank helps you to see the wells

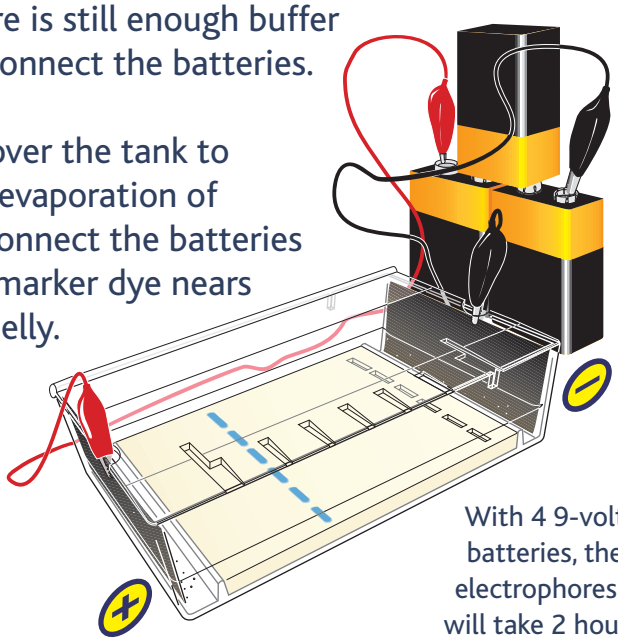
Put the electrodes in place at either end of the tank.

Some of the buffer will soak into the electrodes.



Check that there is still enough buffer over the jelly. Connect the batteries.

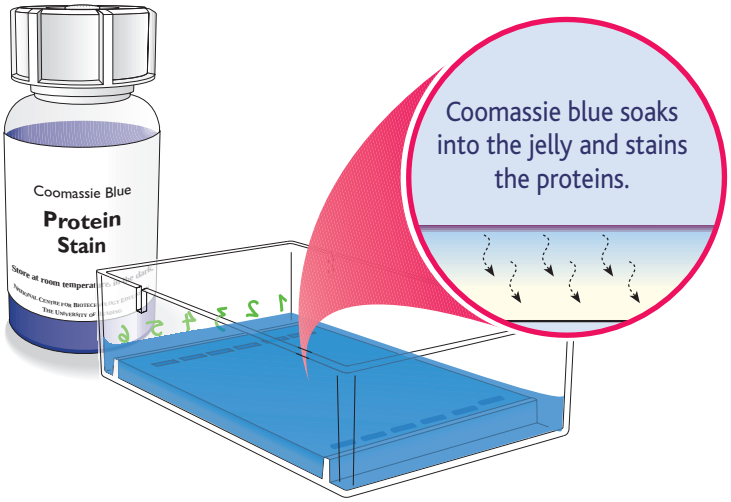
Use the comb over the tank to slow down the evaporation of the buffer. Disconnect the batteries when the blue marker dye nears the end of the jelly.



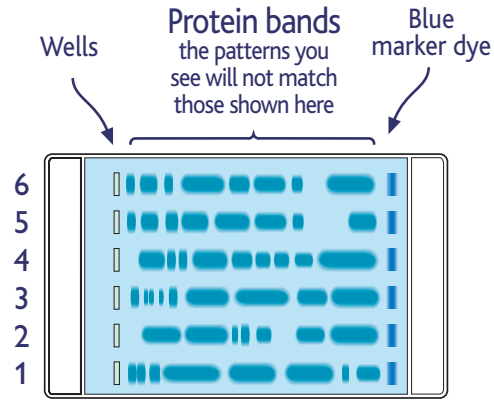
USE NO MORE THAN 36 VOLTS

With 4 9-volt batteries, the electrophoresis will take 2 hours.

Remove the electrodes. Pour away the buffer. Pour Coomassie blue into the tank and leave it for 30 minutes.



Pour off the Coomassie blue. Now pour on destaining solution. Leave the tank overnight, so that excess blue stain in the jelly is washed away. This will leave just the blue protein bands.



Dean Madden