

Protein power!



Teacher's guide





If you need help or wish to comment on this practical kit...

IMPORTANT

The *Protein power!* module is designed to be used with the NCBE modular electrophoresis system. You will need electrophoresis tanks, combs and other items from the Base Unit in addition to the consumable items supplied in the module*.

In this booklet, we have tried to anticipate the questions you might have and any problems that may occur when you prepare this practical work. Consequently it might appear rather daunting. However, the practical exercise is fairly simple to prepare and perform.

If you need help, please contact the NCBE at the following address:

The National Centre for Biotechnology Education

The University of Reading
Whiteknights
PO Box 226
READING
RG6 6AP

T : + 44 (0) 118 9873 743

F : + 44 (0) 118 9750 140

E : NCBE@reading.ac.uk

W : www.ncbe.reading.ac.uk

* If you have one of the special *Science Year Protein Kits*, all of these items are included in the box.

Sources of batteries

Inexpensive batteries are available for school use from the Technology Enhancement Programme (TEP). The school supply part of TEP may be contacted at:

Teaching Resources
Middlesex University
Unit 10
Lea Road
WALTHAM CROSS
EN9 1AS

T : + 44 (0) 1992 716052

F : + 44 (0) 1992 719474

W : www.mutr.co.uk



As this booklet went to print, the NCBE was also planning to supply an inexpensive power pack for use with its electrophoresis equipment. Please contact the NCBE for details.

About protein electrophoresis

What is it?

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

Electrophoresis can be used to compare proteins from different sources.

How does it work?

The molecules to be separated must have an electrical charge. In this practical procedure, the proteins are mixed with Laemmli buffer, which gives them a negative electrical charge.

The charged protein samples are placed in slots or 'wells' at one end of a slab of gel. The Laemmli buffer is very dense, so the proteins are carried with the buffer to the bottom of the wells.

A current is applied and the molecules move out of the wells and through the gel towards the electrode of the opposite charge. A blue dye in the Laemmli buffer shows the progress of the electrophoresis.

Large molecules move slowly through the porous gel; small molecules move more quickly. In this way the molecules 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 key techniques used by molecular biologists and forensic scientists today. As the Human Genome Project nears completion, the study of proteins — the product of all the DNA — is becoming increasingly important.

Why proteins?

There are many different sorts of proteins of widely-differing sizes. Proteins form patterns on gels which can easily be distinguished. Proteins are easy to extract from everyday materials and provide scope for numerous practical investigations and stimulating contexts.

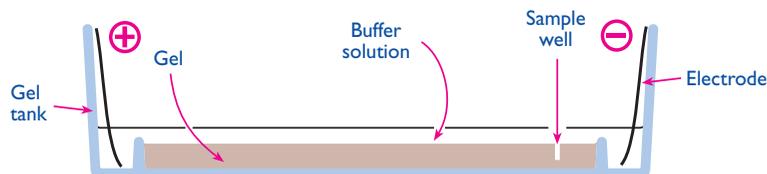
Curriculum links

This work ties in with several areas of the science curriculum. In addition to developing investigative skills, this work can be used to support teaching about:

- Nutrition (referring, for example, to the structure or sources of proteins);
- Variation, classification and evolution;
- The separation of mixtures.

Age and ability range

This practical work is suitable for older Key Stage 3 pupils. With appropriate adaptation, it has also been used successfully with much younger and older pupils.



Preparing, running, staining and destaining a protein gel takes a long time. Careful planning will make this practical activity simpler to undertake.

Cast the gels in advance

Prepare the agarose solution and cast the gels in advance. This will save time during the lesson and should lead to better, more consistent results from the class. Once set, gels can be covered with a little TB/SDS buffer solution and stored in plastic bags in a fridge for up to a week.

Ensure that the students are prepared

Set the context for the work (see 'Investigations', page 9, for ideas) and explain the procedure to the students before the lesson in which you will carry out the work. You could download the overhead masters from the NCBE Web site to help you do this, or ask the students to read the *Student's guide* in advance. Asking the students to choose and bring in suitable protein samples could also form part of the preparation.

Preparing and loading the samples

Proteins can be extracted and the gels loaded and set running within 40 minutes. When the gels are 'run' at the recommended 36 volts, and a fresh set of cells is used, electrophoresis takes about 2.5 hours. Our tests suggest that you can achieve a single run from a set of zinc chloride cells, but three runs from a set of similar alkaline cells. The first two runs with alkaline cells take 2.5 hours each; the third run takes about three hours. Although a lower voltage of 27 volts will also give reasonable results, voltages below this will not be successful.

Staining and destaining the gels

After electrophoresis, the Colloidal Coomassie Blue stain should be left on the gels overnight (about 18 hours at the most). This will colour both the proteins and the agarose gel (Coomassie Blue was originally developed as a blue dye for wool).

To remove the background stain in the agarose, the gels must then be destained using water.

Apart from the lengthy wait, both staining and destaining take only seconds to perform and can be done by a teacher or technician.

The destained gels should be kept in plastic bags in a fridge to prevent them from drying out before they are viewed by the students.

The gels are safe to handle and students may keep them if they wish. Gels should be stored in tightly-sealed plastic bags, in a fridge.



The following equipment and materials are needed by each person or group

- Agarose solution, 3% in TB buffer, ~12 cm³ per gel

Important: This must be a high-quality agarose, suitable for running protein gels. Agarose used for DNA gels is not generally suitable.

- TB/SDS running buffer with 0.1% SDS, ~15 cm³ per gel
- Laemmli buffer; 0.5 cm³ per sample
- Colloidal Coomassie blue stain, ~25 cm³ per gel

- Distilled water for destaining, ~50 cm³ per gel
- 1 cm³ syringe with silicone rubber tubing adapter for loading protein samples into gel
- disposable plastic tips to fit syringe (a new tip is needed for each protein sample)
- 1.5 cm³ microcentrifuge tubes, 1 for each type of protein tested
- Gel electrophoresis equipment and power supply (NCBE gel tank with a 6-toothed comb with 4 × 9 volt PP3 batteries)
- Permanent marker pen for labelling tubes and gel tanks

The following items might also be needed:

- a small rectangle of plastic foam with small holes cut out of it with a cork borer — this is useful for holding the sample tubes on the bench or floating them in a water bath;
- access to a water bath or incubator, set at 60 or 65 °C (for keeping the agarose molten).

For electrophoresis of fish proteins

- Fresh fish samples, a piece about half the size of a grain of rice of each type. Fish proteins deteriorate quickly, and frozen

samples tend not to produce well-defined bands.

For electrophoresis of plant proteins

- Seeds e.g., dried chick-peas, beans, lentils, wheat, barley. Dried green and white French beans are particularly easy to work with.
- Mortar and pestle

N.B. You may need to avoid using beans and other seeds if students in your class are allergic to them.





Tris-Borate (TB) buffer

The solution provided in the kit is a 10x concentrate. Before use, 1 volume of this solution should be diluted with 9 volumes of distilled or deionised water. We recommend that you make up the whole solution in one go, then store the buffer at room temperature until you need it.

Note: If solid materials crystallise in the TB buffer during storage, this will not usually affect the performance of the buffer, as this material only represents a small proportion of the total solutes. If desired, the diluted buffer may be heated gently to re-dissolve this material, but this is not strictly necessary.

Agarose gel (3%)

For reasons of safety and convenience we suggest that the gels are cast by a teacher or technician in advance. Tanks containing cast gels should be tightly wrapped (e.g., in plastic food wrap) to stop them from drying out. They can be stored in a fridge for up to a week.

It may not look much, but this powder is the most expensive item in the kit! 6 g of high-grade agarose is provided. Add the agarose powder to the 200 cm³ of diluted (1x) TB buffer. Heat in a boiling water bath or microwave oven to melt the agarose. Less than a minute at full power in a 940 watt oven will melt 100 cm³ of gel. The container used to hold the molten agarose *must not be sealed*, but lightly covered with plastic film that has been punctured with

one or two small holes. Swirl the gel half-way through the heating cycle to ensure that it is thoroughly mixed.

Once molten, the agarose solution can be kept in this state at 55–60 °C in a water bath. Alternatively, the gel may be kept until needed in a sealed container (e.g., a screw-topped jar). Before use, the gel will have to be melted again by standing it in a boiling water bath or by using a microwave oven as before. Ensure that the agarose solution is mixed well before using it.



Running buffer (used over gel)

Use the remaining 300 cm³ of 1x TB buffer (made as described above) but add the 3 cm³ of 10% SDS solution to it. The running buffer should be kept at room temperature until it is needed. It can only be used once.



Laemmli buffer

(called 'blue marker dye' in the Student's guide)



Laemmli buffer can be used straight from the bottle. It should be stored at room temperature. It contains SDS (a detergent which gives the proteins a negative charge), bromophenol blue dye (so that the samples can be seen easily when loading and tracked during electrophoresis) and glycerol (to make the liquid dense so that the samples can be loaded into the gel).

Colloidal Coomassie Blue

IMPORTANT You must add 10 cm³ of 20% NaCl solution to every 100 cm³ of this stain before use. The stain solution should be stored at room temperature. Shake the bottle well before use! This Coomassie Blue solution cannot be re-used.

Note: This is an adaptation of the method commonly used for staining polyacrylamide gels. This aqueous form of Colloidal Coomassie Blue is safe to handle and should not be confused with the methanol and acetic acid-containing variety which is sometimes used for staining protein gels.



+

SALT

"Oh dear... I've forgotten to add the salt!" If this happens to you, don't panic. The stain will still work, but the gels will require prolonged destaining (i.e., for several days) in water.

If you would like the complete recipes

Recipes for all these solutions are provided on the NCBE's Web site, should you wish to make up your own reagents from scratch:

www.ncbe.reading.ac.uk

General guidelines

In the practical investigation described here, we have tried to check that recognised hazards have been identified and that suitable precautions are suggested.

Where possible, the proposed procedures are in accordance with commonly-adopted general risk assessments. If special precautions are necessary, this has been indicated below.

However, users should be aware that errors and omissions can be made. Therefore, before starting any practical activity, you should always carry out your own risk assessment. In particular, any local rules issued by employers or educational authorities MUST be obeyed, whatever is suggested here. It is assumed that:

- practical work is carried out in a properly equipped and maintained science laboratory;
- any mains-operated equipment is properly maintained;
- care is taken with normal laboratory operations such as heating substances;
- good laboratory practice is observed when chemicals or living organisms are used;
- eye protection is worn whenever there is any recognised risk to the eyes;
- pupils and / or students are taught safe techniques for activities such as handling chemicals.

Specific guidelines

- 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. Hot, molten agarose can scald. It must be handled with care, especially as it is being

taken from a microwave oven. Heat-proof gloves are a sensible precaution.

- The carbon fibre electrode tissue used with the NCBE gel electrophoresis equipment may release small fibres, which can cause minor skin irritation if you handle the tissue a lot. It is a wise precaution to wear protective gloves if you find the tissue unpleasant to handle. However, the fibres released are too large to enter the lungs, so it is not necessary to wear a face mask. In addition, the fibres are soluble in body fluids and are completely biodegradable.
- The NCBE 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. Serious or lethal electrical shock could result if the equipment is connected directly to a mains electricity supply.
- Rechargeable batteries are unsuitable for this work as they can generate potentially dangerous currents and can be damaged if connected in series.
- Colloidal coomassie blue stain presents no serious safety hazard, 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.
- When diluted and used as directed, TB/SDS buffer presents no serious safety hazards. Spent buffer can be washed down the drain.
- When used as directed, the Laemmli buffer presents no serious safety hazards. Used Laemmli buffer can be washed down the drain.

More safety information

Additional relevant safety information can be found in:

Topics in Safety (2001) Association for Science Education. Third Edition. ISBN: 0 86357 3169.

A nice piece of Wensleydale?

Compare cheese samples of different ages. Do vintage, mature and mild cheddars, for example, give different protein 'fingerprints'? What do you think is happening to the proteins in cheese as it ripens?

What's in a 'crabstick'?

Is there really crab protein in a 'crabstick', or is it something fishier? Try running some crabstick protein alongside a variety of proteins that you think might be used to make 'crabsticks' (e.g., fish, soya — or even, er, crab).

The germination game

Germinate some seeds (e.g., beans). Extract proteins from the seeds at different stages of the germination (e.g., daily) and compare the protein patterns you find. What proteins are likely to be produced by germinating seeds?

N.B. You may need to avoid using beans and other seeds if students in your class are allergic to them.

Relative values

Compare proteins from the muscle of fast-swimming fish (such as mackerel or tuna) with their more sedate cousins (plaice or other flat fish).

The raw and the cooked

Compare the way in which raw and cooked proteins behave in a gel (for example, using egg white). What does their movement in the gel suggest about the effect of cooking on the proteins' structure?

Shapeless?

How does the shape of proteins affect the way they run in a gel? Compare proteins extracted from legumes (e.g., peas and beans) which are mostly globular, to those from grains (e.g., wheat, rice and barley) which tend to be more linear.

N.B. You may need to avoid using beans and other seeds if students in your class are allergic to them.

Seafood diet

Compare the proteins from fish muscle with those from invertebrate muscle (e.g., prawns, scallops, etc.). What differences can you detect? How can these observations be explained?

Food forensics

A recent Trading Standards investigation in Lincolnshire found that over 40% of take-away pizzas that were supposed to contain ham and/or cheese did not. Instead, the 'cheese' was a cheaper vegetable oil substitute and the 'ham' was actually turkey, coloured and flavoured to look like ham. Protein electrophoresis could easily be used to check the authenticity of pizza components.

Detergent detective

Washing powders contain a variety of enzyme products; amylases, proteases, lipases and sometimes cellulases. These enzymes are encapsulated in small 'beads' that can be separated from the powder. Most of the bead is insoluble crush-proof packaging (made from cellulose), with the enzyme being at the centre. However, the beads break up in water, releasing the enzyme. Protein electrophoresis can be used to test which enzymes are present in different washing powders. Samples from washing powders can be tested against the pure enzymes, which are available from the NCBE.



Fitting tips onto the syringes

You must use silicone rubber tubing as supplied in the kit. Aquarium tubing will not do; it is simply not elastic enough to hold the disposable tip firmly in place. You need a surprising amount of tubing on each syringe. About 10 mm is required. It must cover the whole of the tip of the syringe and protrude beyond it by about 2 mm to ensure that the disposable tip can easily be placed on and gripped securely.



Loading the gel

First, put each tank where you are going to 'run' it. Once the samples are in the wells, you won't be able to move the tank easily (the chances are that the samples will spill out of the wells if you pick the tank up).

Place a piece of dark paper or card beneath the gel tank, so that the wells show up.

Hint: You can stick a piece of black tape under the tank where the wells are to give a permanent 'loading area'.

Draw up the sample liquid from the top of the tube, leaving any solid bits behind. Wipe the tip on the tube to get rid of excess sample.

You need about 10 mm of the blue liquid in the tip. Not all of this liquid will fit in the well.

When you load the gel, put the tip of the syringe into the buffer solution over the gel. Hold the tip so that it is *just over* one of the wells. There's no need to place the tip in the well itself.

As you press the plunger in, the blue liquid will drop neatly into the well. The marker dye is heavy, so it simply drops in. Don't press the plunger too sharply, or you'll squirt the sample into the well and out again.

Don't worry if some of the sample washes out of the well. It is probably a good idea to slightly overload the wells so that the marker dye (which tends to diffuse into the gel) remains clearly visible by the end of the electrophoresis.

Hint: A good way of demonstrating to students how they should load gels is to place a tank on an overhead projector. The wells and the liquid being pipetted into them will be clearly visible on a screen.

If students pour gels themselves, an OHP can also be used to show how this should be done.

Melting agarose

Ensure that the container used to prepare the agarose gel is clean. Tiny flecks of dust will not affect the way the gel runs, but they can prove a nuisance when you are trying to see faint bands in the gel.

For convenience, dissolve and melt the agarose using a microwave oven. Less than a minute at full power in a 940 watt oven is sufficient for 100 cm³ of gel. The container (flask or beaker) used to hold the molten agarose must not be sealed, but covered lightly with plastic film that has been punctured with one of two small holes. Swirl the gel half way through the heating cycle to ensure that it is mixed thoroughly.

Agarose gel can also be prepared using a hotplate. If this is done, the gel must be swirled as it melts, to prevent charring. Better still, stand the container of agarose in a saucepan of boiling water. The use of a Bunsen burner to melt agarose is *not* recommended.

Once melted, the gel may be kept in a molten state by standing the container in a water bath at 55–60 °C until the gel is needed. Take care when handling the molten gel; it will be very hot, and can scald. The gel should be allowed to cool before it is poured.

The gel takes too long to run

With four new 9 volt batteries the sample should move about 5 mm in 15 minutes. Lower voltages are not recommended.

If, after the first 10 minutes, the dye does not seem to have moved and bubbles are not visible at the cathode, check the electrical contacts between the batteries and the electrodes. Ensure that there is enough buffer above the gel to cover the plastic ridges at each end of the tank, but not so much that most of the current passes through the buffer solution rather than the gel.

If you are used to running DNA gels, you may be surprised by the bubbles produced in the TB/SDS running buffer. This is quite normal (it's due to the SDS detergent), but keep a careful watch to ensure that the bubbles do not prevent contact between the running buffer, gel and electrodes (add more running buffer to the tank if necessary). Remember that during long runs or in a warm environment liquid may evaporate from the buffer. The tank should be covered with the comb to reduce such evaporation.

Note: Unlike the TBE buffer used for DNA gels, the TB/SDS running buffer used for these protein gels cannot be re-used.

Corrosion of crocodile clips

The crocodile clip used at the anode may corrode due to electrolysis. This can discolour the buffer solution and gel, but will not interfere with the electrophoresis.

N.B. Corrosion can be avoided by ensuring that only the first two teeth of the clip are in contact with the electrode tissue, as shown in the picture on the right.

Viewing the stained gel

The bands are seen most easily by holding the gel at an angle against a well-lit surface. A light box is ideal, but if one is not available, you can put a sheet of white paper on an overhead projector, and put the stained tanks on that for direct observation.

There's no protein on the gel

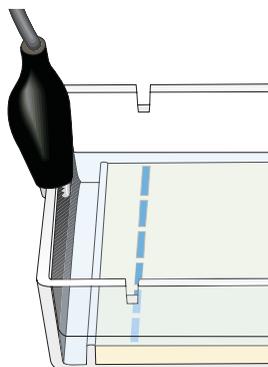
The most common cause of this problem is poor loading of the sample. Sometimes insufficient material has been placed in the well. Alternatively, students may have punctured the well with the tip while loading the sample. In such cases, the sample will have moved beneath the gel without separation.

There's a large smear on the gel, but no distinct bands

Here, the most likely cause is too large a protein sample being used. A piece of material no larger than half a grain of rice is needed.

The protein bands are very faint

It is possible that the staining procedure has not been followed correctly. After a few days, you may find that the colour of the bands intensifies, particularly if the background stain from the Coomassie Blue was very strong to start with. Fresh protein samples (e.g., fresh fish rather than frozen) will give better results.



The NCBE's Web site has numerous resources to support this kit. You may also find the following Web sites of interest. They vary in complexity and not all of them are suited to schools use.

National Centre for Biotechnology Education

<http://www.ncbe.reading.ac.uk>

This site includes additional information and resources to support this kit, including video clips and OHP masters that can be downloaded free-of-charge.

Science and Plants for Schools

<http://www.saps.org.uk>

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

Biotechnology and Biological Sciences Research Council

<http://www.bbsrc.ac.uk/schools>

Schools pages of the body that funds research and training in the non-medical life sciences in the UK.

Protein Data Bank — Myosin

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

Information about one of the two main proteins in muscle ('Molecule of the month' series).

Protein Data Bank — Actin

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

Information about one of the two main proteins in muscle ('Molecule of the month' series).

Folding@Home

<http://folding.stanford.edu/>

Use your computer to help in the international research effort to find out how proteins fold.

RASMOL (molecular modelling software)

<http://www.umass.edu/microbio/rasmol>

Display protein structures in many different formats using this free software, which is available for Macintosh, Windows and a variety of other operating systems.

ASE Science Year CD-ROMs

<http://www.sycd.co.uk>

This Web site carries the CD-ROMs that have been produced for the UK's Science Year in 2002. CD 1 includes the original NCBE Protein electrophoresis protocol.

The challenge of how proteins fold

http://www.the-scientist.com/yr1998/oct/bunk_p1_981026.html

A paper in 'The Scientist' on-line magazine.

Unravelling the mystery of protein folding

<http://www.faseb.org/opar/protfold/protein.html>

A general introduction in FASEB magazine.

Database of interacting proteins

<http://dip.doe-mbi.ucla.edu/>

A list of protein pairs that are known to interact with one another.

Expert Protein Analysis System (ExPASy)

<http://www.expasy.org/>

Numerous resources, mostly aimed at researchers, but including an art gallery, a monthly 'Spotlight on proteins', and some rather less than amusing Swiss protein jokes.

Please note: The NCBE Web site will maintain a list of up-to-date links and new sites will be added as we discover them.

A wide variety of people and organisations helped this Protein Electrophoresis Kit become a reality. We're delighted to thank everyone concerned here.

The practical activity in this kit is based upon one developed by the **National Centre for Biotechnology Education** (The University of Reading) and **Science and Plants for Schools** (Homerton College, Cambridge). The origin of this work lies in *'The Fishy Fish Finger'*, a protocol that was developed by the NCBE with our colleagues **Margareta Wallin** and **Elisabeth Strömberg** at Göteborg University.

Peter Finegold of The Wellcome Trust is guilty of kick-starting the current initiative however — it was he who encouraged us to develop an activity for the UK's *Science Year* in 2002 and tried to bring the interested parties together. **Erica Clark** and more recently, **Paul Beaumont** of SAPS were supportive beyond the call of mere duty. Quite simply, this initiative would not have been so effective without their unstinting and generous-spirited backing.

The practical activity was tested by students at Roundwood Park School, Hertfordshire (see photographic evidence) and both **Leighton Dann** (formerly a teacher at that school) and **Roger Delpuch** (of The Haberdashers' Aske's Boys' School) were instrumental in shaping the methods described here.

Chantelle Jay of the Biotechnology and Biological Sciences Research Council was enthusiastic and gave scientific and practical support. She also very kindly hosted one of the early meetings that led to the development of this kit.



The Association for Science Education kindly distributed the original version of the practical protocol on its first *Science Year* CD-ROM. Both **Daniel Sandford-Smith** and **Jenifer Burden** of the Association proved exceptionally patient while these materials were being written.

The gel electrophoresis equipment used for this work was developed by **Dean Madden** and **John Schollar** at the NCBE. John did much to refine the original protein electrophoresis method, greatly improving its safety and ease-of-use.

The documents in the kit were written and illustrated by Dean Madden, although it will be apparent that the ideas described have benefited from the contributions of many people.

John Tranter of CLEAPSS and **John Richardson** of SSERC and their colleagues gave advice on electrical safety, for which we are grateful.

Our colleagues in the **School of Food Biosciences** at The University of Reading emptied several rooms and moved numerous boxes so that we had somewhere to store the materials that are in this kit. 40,000 booklets and 50,000 microcentrifuge tubes occupy a lot of space...

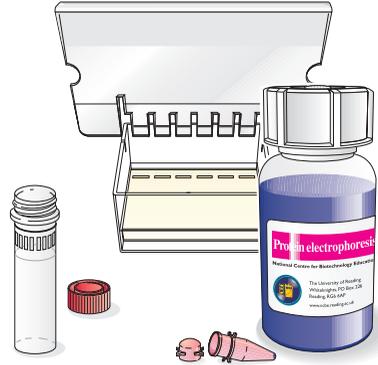
Finally we should like to express our gratitude to **The Royal Society** and the **Gatsby Charitable Foundation** for their support of the *Science Year Protein Kit* during the UK's *Science Year*.

Replacement items

The NCBE supplies a complete range of refill packs and consumables for the various electrophoresis kits, including:

Dried Lambda DNA
Dried restriction enzymes: *Bam*HI, *Eco*RI, *Hind*III
Gel tanks
4-tooth combs
6-tooth combs
Microsyringes and calibrated microsyringe tips
Carbon fibre electrode material
High-grade agarose
TBE, TB, TE, Extraction and Phosphate buffers
Non-toxic Azure A DNA stain
Non-toxic Coomassie Protein stain
Lambda DNA module
Nature's dice module
Protein electrophoresis module

Full colour *Students' Guides* from the various NCBE electrophoresis modules.



For information, help or to place an order

You can telephone, fax or write to us:

The National Centre for Biotechnology Education
The University of Reading
Whiteknights
PO Box 226
READING
RG6 6AP
The United Kingdom

T : + 44 (0) 118 987 3743
F : + 44 (0) 118 975 0140
E : NCBE@reading.ac.uk
W : www.ncbe.reading.ac.uk



We can usually accept credit card orders (*VISA* or *MASTERCARD*). If you order from educational institution and require an invoice, please provide an official order number.

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