

# Protein electrophoresis

- **What is it?**

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

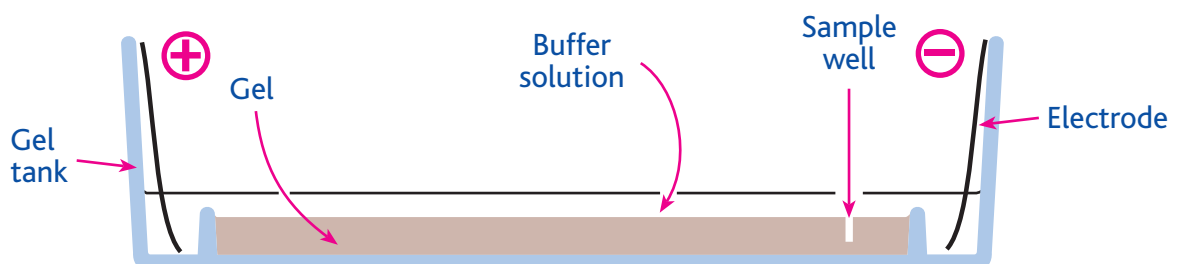
- **How does it work?**

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 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 or DNA 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. Without it, the human genome project would have been impossible and genetic fingerprinting could not be done.

- **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.

# Protein electrophoresis

## ■ Protein gel electrophoresis

Electrophoresis can be used to compare proteins from different sources. A highly-refined agarose gel is used here because polyacrylamide gels (which are often used for this type of work) are hazardous to prepare.

## ■ 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 protein structure)
- 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. A reasonable degree of manual dexterity and good eyesight is required to load electrophoresis gels.

## ■ Timing

If the gels are prepared (cast) in advance, proteins can be extracted and the gels loaded and set running within 40 minutes. It takes between 2 and 12 hours to run the gels, depending upon the voltage used. After electrophoresis, stain should be left on the gels for 20–30 minutes, which should then be destained overnight.

## ■ Advance preparation

For reasons of safety and convenience we suggest that 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.

## ■ Suppliers

A special pack containing all of the equipment and materials needed can be obtained from **The National Centre for Biotechnology Education**:

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

Molecular biology suppliers such as **Bio-Rad** also provide an extensive range of equipment for protein electrophoresis:

<http://explorer.bio-rad.com>

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  
Technology Education Centre  
Trent Park  
Bramley Road  
LONDON  
N14 4YZ  
T : 020 8 447 0342  
F : 020 8 447 0340

## ■ Equipment and materials

Needed by each person or group

- Agarose solution, 3% in TB buffer (~ 12 ml is needed if you use the NCBE electrophoresis equipment)  
*Important: This must be a high-quality agarose, suitable for running protein gels, e.g., 'Agarose 1000' (Invitrogen/Life Technologies) or 'Amplisize' (Bio-Rad).*
- TB running buffer, 15 ml
- Laemmli buffer, 0.5 ml per sample
- Coomassie blue stain, ~ 25 ml
- Destaining solution, ~ 25 ml
- 1 ml syringe with silicone rubber tubing adapter and tips or a similar device, for loading protein samples into gel (a new tip is needed for each protein sample)
- 1.5 ml microcentrifuge tubes, 1 for each type of protein tested
- Gel electrophoresis equipment and power supply (e.g., NCBE gel tank with a 6-toothed comb with 4 x 9 volt PP3 batteries)
- Permanent marker pen for labelling tubes and gel tanks

For electrophoresis of fish proteins

- Fresh fish samples, about 0.5 g of each type.  
Invertebrate (shellfish) muscle has different proteins to vertebrate muscle. In addition, differences may be observed between the muscle proteins of fast-swimming and bottom-dwelling species. 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

## ■ Safety

### General guidelines

In the practical investigations 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.
- 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.
- Gels should be destained in a fume cupboard, as the fumes from the destaining solution are unpleasant.
- When diluted and used as directed, TB buffer presents no serious safety hazards. Spent buffer can be washed down the drain.
- When used as directed, the loading dye presents no serious safety hazards. Used loading dye 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.

#### ■ **For more information**

The following Web sites will have useful additional resources during Science Year:

##### **Bio-Rad Laboratories**

<http://explorer.bio-rad.com>

##### **The Biotechnology and Biological Sciences Research Council (BBSRC)**

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

##### **The National Centre for Biotechnology Education (NCBE)**

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

##### **Science and Plants for Schools (SAPS)**

<http://www-saps.plantsci.cam.ac.uk>

The Protein Data Bank has structure data and information about thousands of proteins:

<http://www.rcsb.org/pdb/>

The on-line journal **Bioscience Explained** also has more advanced information about protein electrophoresis:

<http://www.bioscience-explained.org>

## ■ Recipes

### Tris-Borate (TB) buffer

Makes 1 l

- 10.9 g Trizma base
- 5.56 g Boric acid

Add the above ingredients to 950 ml deionised water.

If necessary, adjust the pH to 8.5 with 1M NaOH.

Make up to 1 litre with distilled or deionised water.

### Agarose gel (3%)

Makes 100 ml

- 3 g protein electrophoresis-grade agarose
- 100 ml TB buffer

Add the agarose powder to the 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 ml 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 halfway 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. Ensure that the agarose solution is mixed well before casting gels.

### Laemmli buffer (called 'blue marker dye' on students' sheets)

Makes 100 ml

- 6.25 ml 1M Tris HCl, pH 6.8
- 20 ml 10% SDS solution
- 25 ml glycerol
- 10 ml 0.1% aqueous Bromophenol blue

Mix the above ingredients well, allow to settle and make up to 100 ml with distilled or deionised water.

### Coomassie blue stain

Makes 100 ml

- 0.02 g Coomassie blue
- 50 ml IMS (industrial methylated spirit)
- 10 ml glacial acetic acid

Dissolve the Coomassie blue in the IMS. Add 40 ml of distilled or deionised water. Add 10 ml glacial acetic acid, and mix well.

### Destaining solution

Makes 1 l

- 200 ml ethanol (or IMS)
- 50 ml glacial acetic acid
- 750 ml deionised water

Mix the ingredients listed above together.