

## APPLICATION NOTE - ELECTROPHORESIS

CONSORT bvba, parklaan 36, B2300 Turnhout, Belgium  
tel:+32/14/411279 • fax:+32/14/429179 • e-mail: [support@consort.be](mailto:support@consort.be)

### POWER SUPPLIES

#### What are the relations between Voltage, Current, Power and Resistance?

Power (W) = Voltage (V) x Current (A)

Resistance ( $\Omega$ ) = Voltage (V) / Current (A)

#### How does a power supply react after pressing RUN?

The internal generator will start building up the high voltage at the output terminals while voltage and current are constantly measured and power calculated. When one of the pre-set parameters is exceeded, the generator stops and will keep that parameter constant.

#### How important is the resistance of an electrophoresis unit?

The resistance of an electrophoresis unit depends on its size, gel thickness, amount of buffer, buffer conductivity and temperature. This resistance will normally decrease in time due to a slowly increasing temperature. Electrophoresis units which have a resistance below the minimum load resistance of a power supply will trigger an alarm! Read the output voltage and current during a run to measure the resistance and use above formula to calculate the value.

#### How to keep a constant voltage during a run?

Program the desired voltage and a higher current and power than the maximum expected values:

Current > Voltage / Resistance

Power > Voltage x Current

#### How to keep a constant current during a run?

Program the desired current and a higher voltage and power than the maximum expected values:

Voltage > Current x Resistance

Power > Voltage x Current

#### How to keep a constant power during a run?

Program the desired power and a higher voltage and current than the maximum expected values:

Voltage > Current x Resistance

Current > Voltage / Resistance

#### Why are my output values different from those of a similar experiment?

Either your programmed parameters are not equal to those described or the resistance of your electrophoresis unit is different (see above). It cannot be due to e.g. an other model of power supply as the relations between Voltage, Current, Power and Resistance are monitored in the same way by any instrument (the electrical laws cannot be disregarded!).

#### What about connecting more than one unit to the same power supply?

The outlets being in parallel each electrophoresis unit will be supplied with exactly the same voltage. However, current and power may differ due to differences between them even when exactly the same model, gel, buffers, etc... are used. Therefore, it is recommended to run several electrophoresis units only in the constant voltage mode on the same power supply.

## HORIZONTAL GEL ELECTROPHORESIS

### Gasket too tight or too loose?

Simply remove the gaskets from the ends of the gel trays and refit into the groove. Refit with the gasket protruding slightly from the ends if the gel tray was too tight or with the gasket fitted below the top edges if the gasket was too loose.

### How to select the gel concentration?

The range of fragment sizes to be separated will determine the choice of agarose concentration for a gel. Typical agarose concentration is 0.5% to 3.0%. For large DNA fragments low-percentage gels are required, while for small DNA fragments, high-percentage gels are recommended. Weak gels (0.5% agarose) should be electrophoresed at low temperatures (e.g.  $-4^{\circ}\text{C}$ ).

Agarose gels of 0.75% to 1.0%, for routine electrophoresis, are recommended for a wide range of separations (0.15 to 15 kb). 2...4% agarose gels are usually selected for PCR fragment resolution. If the gel has to be subsequently photographed, thin gels (2 to 3 mm) with low-percentage agarose are better than thick or high-percentage gels. The latter produce increased opaqueness and autofluorescence.

### How to select the electrophoresis buffer?

TAE buffer provides optimal resolution of fragments  $>4$  kb in length, while for 0.1 to 3 kb fragments, TBE buffer should be selected. TBE has both a higher buffering capacity and lower conductivity than TAE and therefore should be used for high-voltage electrophoresis. Additionally, TBE buffer generates less heat than TAE at an equivalent voltage and does not allow a significant pH drift. Note: because of its lower buffering capacity, TAE should be circulated or mixed from time to time for full-length electrophoresis, especially at higher voltages.

### How to select the enhance resolution?

2 x TAE buffer can be used in units with low buffer volume to enhance resolution during extended runs.

### What about the influence of temperature?

Electrophoresis at high voltages produces heat. Additionally, high-conductivity buffers such as TAE generate more heat than low-conductivity buffers. Care should be taken in agarose gel electrophoresis with voltages greater than 175 V, as heat build up can generate gel artifacts such as S-shaped migration fronts, and in extended electrophoresis runs, can even melt the agarose gel. With high voltage electrophoresis, the use of low-melting-point agarose gels should be avoided.

### What about the DNA mobility?

DNA fragments as small as 1 kb or less can be separated using agarose gel electrophoresis. For fragments smaller than 0.1 kb, polyacrylamide gels are more suited.

### What about the RNA mobility?

Either before or during electrophoresis, RNA should be denatured. For example, RNA fragments which have denatured with glyoxal and dimethyl sulphoxide can be separated on neutral agarose gels, or RNA can be fractionated on agarose gels containing methylmercuric hydroxide or formaldehyde. RNA samples usually require longer runs or buffers that are easily depleted, so it is necessary to circulate the buffer. Northern analyses should not normally be run on a mini gel tank.

### Separation performance?

Gel concentration, running buffer, voltage, temperature, conformation, and the presence of ethidium bromide all affect separation results. To establish progress of double-stranded DNA, ethidium bromide (0.5  $\mu\text{g}/\text{ml}$ ) is often added to running buffer.

The dye's fluorescence properties allows the band to be visualised under a UV lamp. However, ethidium bromide may slow the DNA migration rate by approx. 15%. As an alternative, after electrophoresis, the gel may be stained in an ethidium bromide solution (0.5  $\mu\text{g}/\text{ml}$   $\text{H}_2\text{O}$ ) for 15 to 60 minutes and then viewed or photographed on a UV trans-illuminator.

Note: staining time should be minimised to prevent small nucleic acid fragments from diffusing out of the gel. Background fluorescence of unbound ethidium bromide can be minimised through destaining by soaking the gel for 5 minutes in 0.01 M  $\text{MgCl}_2$ , or for 30 minutes in de-ionised water. Caution! Ethidium bromide is a known mutagen. Always wear gloves when handling. Wear UV safety goggles and protect skin when using any UV light source.

## VERTICAL GEL ELECTROPHORESIS

### How to prevent leaking gels?

The two most important things to be aware of when casting gels using the caster systems are:

- a. that the glass plates have been inserted into the casting or gel running module on a flat surface.
- b. that the spacers are flush with the bottom edges of the glass plates.

Perfect alignment of spacers can be guaranteed using the new glass plates with bonded spacers.

### How to overcome polymerisation problems?

If you are experiencing problems obtaining good polymerisation adjacent to spacers and combs then this can be overcome by pre-soaking the combs and spacers in distilled water or a 10% solution of ammonium persulphate.

### How to avoid over-tightening?

Over-tightening the cam pins on the casting systems is a common cause of problems when using these units. Cams should only be tightened just until appreciable pressure is felt.

### How to extract the tube gel?

Tube gel electrophoresis can be difficult because of problems with extracting the tube gel from the capillary tube. The tube gel is best extracted by gently pipetting liquid behind the tube gel and then catching it in the Gel extraction platform.

### How to enhance transfer?

If the gel blot sandwich is too thick, this may bow the cassette causing loss of contact between gel and membrane resulting in poor transfer. The thickness of the blot can be lessened by removing the fibre pad on the non-membrane side of the blot.