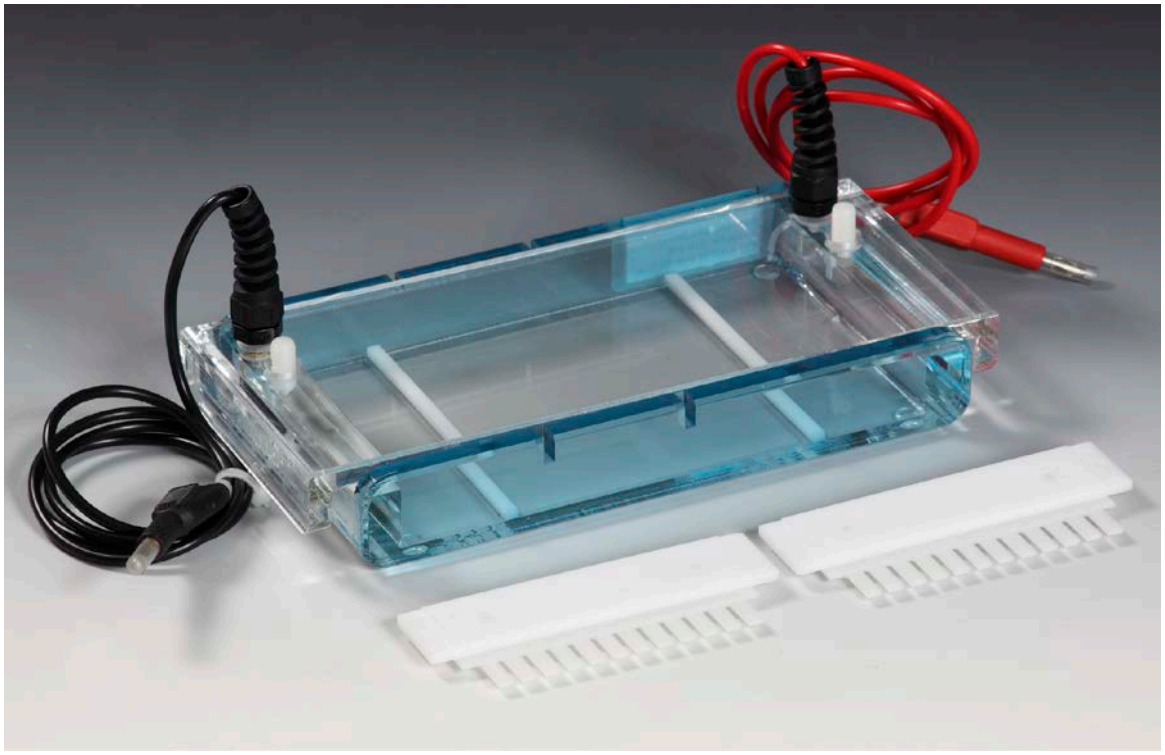
Instruction Manual
Elektrophoresis Chamber „Mini“
Art.No. EK-100



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Scope of supply

The model „Mini“ contains the following items:

- Electrophoresis chamber with corrosion-resistant platinum electrodes and high-quality connections for plus and minus pole
- Safety lid with attached power cords
- 2 combs, 1.5mm thick, 12 teeth each
- Instruction manual

Safety instructions

- Please read the instruction manual carefully before using the electrophoresis system.
- Use only CE-marked power supplies.
- Always disconnect the electrophoresis chamber from the power supply before removing the safety lid.
- Do not put down the safety lid on wet surfaces.
- Always disconnect the electrophoresis chamber from the power supply when not in use.
- The chamber is provided for a max. current and voltage. Do not exceed 150 mA, 120 V.
- Please mind the maximum filling level for the electrophoresis buffer.

Technical properties

The horizontal electrophoresis system “Mini” has been designed in a way that allows to cast and run the gel directly in only one chamber. The user does not need any additional casting equipment, such a grease or seal, to prepare the gel tray for casting the gel.
The chamber’s bottom is UV-permeable, thus the gel run can easily be documented in case of using DNA-fluorescent dyes. Self-evidently, the gel can also be removed from the chamber for staining and documentation after the gel run.
Please use only UV light with a wave length of 300nm or higher for all applications, as hard UV light (<300 nm) damages the gel chamber and the nucleic acids.
If you need only short runs, you can use both combs. Then you have twice as many slots for samples available.

Warranty

The manufacturer guarantees that the electrophoresis chamber was carefully tested before delivery and that it complies with the applicable safety regulations.
Please check the delivery immediately upon receipt for completeness and possible damages in transit. If the supply is faulty or damaged, please contact the service of 3B Scientific immediately.
The manufacturer gives a 24-months warranty on the product if the chamber has been used according to the instruction manual. Claims for replacement or repair do not exist if physical abuse is the reason for the damage. Any liability for conseqential damages resulting from the use of the electrophoresis chamber is hereby excluded.The manufacturer's liability for intent and gross negligence or for damages resulting from injury to life, body or health is unaffected by that.
According to experience, the two platinum electrodes last for many years if the product is used properly. Damage occurs mostly due to mechanical destructions (e. g. by washing-up brushes) or contact with so-called “platinum poisons” such as phosphorus, boron and heavy metals (lead, zinc, etc.). For this reason the platinum electrodes are excluded from the manufacturer’s warranty. However, the manufacturer is willing to repair damaged platinum electrodes or other damage to the electrophoresis system at a reasonable price, still after the warranty period.

In order to introduce advancement developments promptly, the manufacturer reserves the right to change specifications and small visual details without prior notice.

General instructions

Casting of agarose gels

1. Please wear protective clothing, goggles and gloves for heating the agarose solution.
2. Remove the safety lid of the electrophoresis chamber carefully.
3. For the preparation of the gel solution, only suitable agaroses and electrophoresis buffers should be used. For preparing the agarose solution weigh a corresponding amount of agarose in a small Erlenmeyer flask, add an appropriate amount of electrophoresis buffer and a stir bar (heating with heating stirrers). Note the weight of the filled flask so that emerging cooking losses can be compensated by the addition of (distilled) H_2O. Thus, the gel solution has the agarose concentration that you need. To dissolve the agarose, heat the Erlenmeyer flask either in the microwave or with a heating stirrer. For the latter, use medium heating power and stir constantly. The heating in the microwave should be done only for a short period of time, on medium heating level, and then repeated multiple times. When heating in a microwave, remove the Erlenmeyer flask from time to time (mind wearing gloves, goggles and possibly pay attention to boiling!) and gently swirl the gel solution in a circle. Put it back in the microwave afterwards and repeat the entire process 3-4 times, until the agarose is completely dissolved.
4. Before the casting of the gel, allow the gel solution to cool down to 60°C. Cast the gel solution without any air bubbles in the gap between the two white plastic stripes on the chamber's ground (its capacity is about 40 ml). Then insert the comb (the two combs respectively). Make sure that there are no air bubbles on the edges of edges of the slots. Gels made of standard agarose solidify within 20 minutes at room temperature.
5. After the solidification of the agarose gel, fill some of the electrophoresis buffer in 3-mm layers in the chamber over the gel to prevent the gel from drying out. After carefully removing the comb(s), the gel can now be loaded, The coated gel can be stored for some days at 4°C. For that, best let the comb inserted and cover the gel with wrapping film to protect it from drying out.

Loading of gels and electrophoresis

1. After the solidification of the agarose gel, cover the gel with the electrophoresis buffer. Please note the max. filling level for the electrophoresis buffer.
2. Remove the comb(s) from the agarose gel by gently moving it (them) back and forth and then carefully pulling it (them) out upright.
3. In the next step, the slots in the gel can be loaded with the samples. Beforehand, the samples have to be prepared with an appropriate gel loading buffer, which increases the specific weight of the samples. Thus, it becomes easier to pour them into the slots with a micropipette (see section "gel loading buffer").
4. When loading the gel slots, dip the tip of the micropipette carefully into the slot and then slowly press out the sample. Make sure not to damage the bottom of the slot. Beginners should practise this with "practice samples" consisting of H_2O and gel loading buffer only.
5. At least one size standard should be applied on each gel so that the size of the various DNA fragments can be determined.
6. Now place the safety lid on the electrophoresis chamber and connect the chamber with a suitable power supply.
   Please note the correct polarity. In an alkaline up to a neutral medium nucleic acids are charged negatively and migrate to the anode (red pole).
7. Turn on the power supply and run the electrophoresis at a reasonable voltage (80 - 120 V). The process of the electrophoresis can be observed with the help of the dye, which is inside the gel loading buffer.
8. Bromophenol blue and xylene cyanol are frequently-used dyes for the gel loading buffer. These dyes are negatively charged, too and migrate to the anode as well. This process,

the so-called co-migration with the double-stranded DNA fragments, depends on different factors, such as agarose type, electrophoresis buffer and gel strength.
For a rough estimation: bromophenol blue migrates in 1xTAE electrophoresis buffer and 1%-standard agarose gel in the same way as a DNA fragment of 650 base pairs. Under the same conditions, xylene cyanol migrates like a fragment with 5 000 base pairs.

Staining of nucleic acids
Since there are different methods of staining nucleic acids, we refer at this point to the corresponding technical literature (Sambrook et. al., 1989).

Evaluation / documentation
Size determinations of nucleic acid fragments can be carried out by comparison with the fragments of a length standard. A detailed description of this procedure can be found, for example, in *Molecular Genetics* by R. Knippers (2008).

Cleaning and maintenance

Attention: Disconnect the electrophoresis chamber from power supply before cleaning.

The gel chamber and the combs should be cleaned with warm water immediately after each use. If necessary, a few drops of a washing-up liquid can be added. Please do not use dishwashing brushes or the like, as this can damage the platinum electrodes. Rinse the electrophoresis chamber after cleaning with distilled or demineralised water to prevent the formation of limescale.
Never leave the chamber uncleaned for hours or even overnight as residues from gel or buffer can cause stains which are difficult to remove completely.
Attention: Please avoid the electrical connections to get in touch with water. If this have happened once, though, please carefully wipe dry with a soft dish / kitchen towel and allow to air-dry. Please do not use paper for the reason that it is often too rough and causes small scratches. Do not use a hair dryer either, as the hot air can damage connectors, fittings and the acrylic material.

Attention: Do neither use ethanol nor other organic solvents for cleaning. It can cause cracks, scratches or other unwanted material changes (blindness etc.).

Required material and recipes

Electrophoresis buffer
The electrophoresis buffer provides the necessary ions for the electrophoresis process. Its addition provides a constant pH value so that nucleic acids have the desired net charge. Nucleic acids are negatively charged in an alkaline up to a neutral medium. Normally, electrophoresis buffer includes certain components that protect nucleic acids from degradation, e.g. EDTA, which complexes divalent cations and therefore inhibits DNases.
At this point, the frequently-used TAE electrophoresis buffer for electrophoresis of DNA is described for non-denaturing conditions. TAE stands for Tris-acetate-EDTA. You can either produce the buffer yourself or order it from 3B Scientific as a prefabricated buffer concentrate.

Agarose, gel volumes and concentrations
Even though various agaroses are offered, the so-called standard agarose is certainly of prime importance. For the casting of an agarose gel in this chamber about 40 ml of gel solution is needed. Please prepare a little more gel solution due to the fact that small residues always remain in the vessel.
Depending on the agarose concentration of the gel, the molecules of different sizes can be separated optimally. The size ranges are listed in the chart below.

Optimal separation ranges of the following DNA length (double-stranded DNA) corresponding to various concentrations of agarose (standard agarose)

concentration of agarose (%)	agarose (g)	buffer (ml)	optimal separation range (kbp)
0,5	0,25	50	1 – 15
0,7	0,35	50	0,8 – 10
1,0	0,5	50	0,5 – 7
1,2	0,6	50	0,3 – 6
1,5	0,75	50	0,2 – 4
2,0	1,0	50	0,1 - 3

Gel loading buffer
The samples which are to be analyzed need to be mixed with a suitable gel loading buffer before applying them to the gel. Gel loading buffer contains dye(s) for tracking the run of the electrophoresis as well as glycerin, saccharose and the like. Therefore, the prepared samples are heavier than the electrophoresis buffer and will decrease slightly into the gel slots during the sample application. Bromophenol blue and xylene cyanol are frequently-used dyes for gel loading buffers. The patterns of their run (or the so-called co-migration to double-stranded DNA fragments) depend on the type of agarose, gel strength and the type of electrophoresis buffer.
For a rough estimation: bromophenol blue migrates in 1xTAE electrophoresis buffer and 1%-standard agarose gel in the same way as a DNA fragment of 650 base pairs. Under the same conditions, xylene cyanol migrates like a fragment with 5 000 base pairs.

DNA size markers
For the sizing of the samples, apply one DNA size standard on each gel at least to one line. DNA size markes are made of DNA fragments of familiar sizes.

Staining of nucleic acids
Since there are different methods of staining nucleic acids, we refer at this point to the corresponding technical literature (Sambrook et. al., 1989).

Declaration of conformity

The electrophoresis chamber „Mini“ complies with the applicable security requirements according to EN: 50081-1 (1992) und EN: 50082-1 (1992).

Literature

Ausubel, Frederik M. et al. (ed.). 2005. *Short Protocols in Molecular Biology, A Compendium of Methods from Current Protocols in Molecular Biology*.

Knippers, R. 2008. *Molekulare Genetik*.

Sambrook J., Fritsch E.F. and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.