

# CUBETA DE ELECTROFORESIS HORIZONTAL HORIZONTAL ELECTROPHORESIS CELL CUVETTE D'ÉLECTROPHORÈSE HORIZONTALE

Referencias | Codes | Références ZFD013, ZFD020, ZFD021, ZFD022, ZFD023



Este manual es parte inseparable del aparato por lo que debe estar disponible a todos los usuarios del equipo. Le recomendamos leer atentamente el presente manual y seguir rigurosamente los procedimientos de uso para obtener las máximas prestaciones y una mayor duración del mismo.

*This manual should be available for all users of these equipments. To get the best results and a higher duration of this equipment it is advisable to read carefully this manual and follow the processes of use.*

*Ce manuel est une partie indissociable de l'appareil et doit être mis à la disposition de tous les utilisateurs de l'équipement. Nous vous recommandons de lire attentivement ce manuel et de suivre scrupuleusement les procédures d'utilisation afin d'obtenir des performances maximales et une plus longue durée de vie de l'appareil.*

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## SAFETY PRECAUTION



WHEN USED CORRECTLY, THESE UNITS POSE NO HEALTH RISK. HOWEVER, THESE UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THIS INSTRUCTION MANUAL.

ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.

THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.

THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.

## MAINTENANCE

### Cleaning horizontal units

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Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60°C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent buildup of salts, but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.

Air drying is preferably before use.

#### **The units should only be cleaned with the following:**

Warm water with a mild concentration of soap or other mild detergent. Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons.

The units should not be left in detergents for more than 30 minutes.

**The unit should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:** Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol.

### RNase decontamination

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This can be performed using the following protocol:

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 10 minutes.

Rinse with 0.1% DEPC (diethyl pyrocarbonate) treated distilled water.

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using.

RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

## SETTING UP THE HORIZONTAL GEL TANKS

### Instructions for fitting electrode cables

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1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit, note if the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible to that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

## Gel preparation

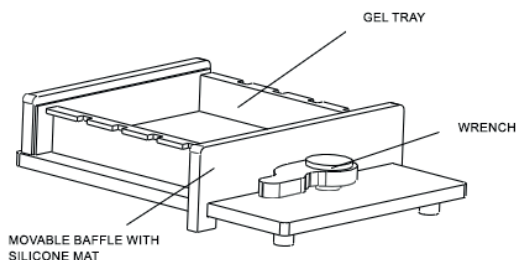
1. For a standard 0.7% agarose gel, add 0.7grammes of agarose to 100ml of 1x TAE or TBE solution. The same 1x solution should be used in the tank buffer solution.
2. Add the agarose to a conical flask.
3. Add the appropriate amount of 1x TAE or TBE solution. To prevent evaporation during the dissolving steps below, the conical flask should be covered with parafilm.
4. Dissolve the agarose power by heating the agarose either on a magnetic hot plate with stirring bar or in a microwave oven. If using the microwave method, the microwave should be set at **400 watt or medium setting and the flask swirled every minute. The solution should be heated until all crystals are dissolved. This is best viewed against a light background. Crystals appear as translucent crystals. These will interfere with sample migration if not completely dissolved. The gel must be cooled to between 50°C and 60°C degrees before pouring.**

## GEL POURING

### Using the gel box:

1. Put the gel box onto a level surface and put a fit gel tray into it. In order to prevent gel leaking, both ends of gel tray must be closely against the gel box.
2. Put the comb(s) onto the tray.
3. Pour agarose carefully so as not to generate bubbles.
4. Leave the gel by itself and wait it to concrete.
5. Pull the comb(s) out carefully and move the tray with gel to the main tank.

### Using the gel casting:



1. Put the gel casting onto a level surface.
2. Put a fit tray into the gel casting and keep both ends of the tray are closely against the silicone mat of the gel casting.
3. Insert the wrench into a appropriate hole according to the size of the tray.
4. Turn the wrench to fasten the gel tray.
5. Put the comb(s) onto the tray.
6. Pour the agarose carefully so as not to generate bubbles. The occurred bubbles can be smoothed and dispersed with a pipette tip.
7. Leave the gel by itself and wait it to concrete.
8. Pull the comb(s) out carefully and move the tray with gel to the main tank.

**Using the cellulose acetate membrane:**

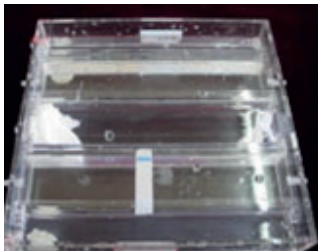
1. Put the membrane into the buffer, full immersed.



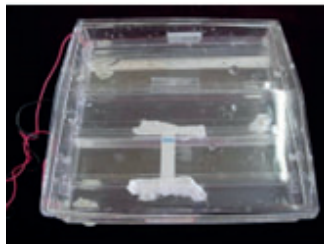
2. If only one sample, cut off a strip of the membrane for use.



3. Dip sample liquid and stamp on the membrane.



4. Put the membrane on the two movable bars across the bridge.



5. Keep two edges of the membrane immersed in the buffer in tank.



6. The movable bar can be adjusted according to the size of cellulose acetate membrane.

#### Running the gel:

1. Mix the sample with buffer- see below solutions for common sample buffers.
2. Pour buffer into the tank till the gel is immersed, almost 1mm higher. Thus, will complete the experiment in shorter time and better quality of sample resolution.
3. Load the samples into the wells with pipettes. Multi-channel pipettes can be used with MC compatible combs to load samples.

Sample loading for cellulose acetate membrane electrophoresis: Dip sample liquid and stamped on the cellulose acetate membrane.

4. Carefully cover the tank with lid and connect it with a power supply.
5. Typically gels run under 90V- 50V. Be noted that, generally higher voltage enables faster electrophoresis but poorer quality of sample resolution.
6. Run electrophoresis.

#### Gel staining and viewing:

1. Put the gel containing the appropriate volume of 0.5ug/ml ethidium bromide to a staining box and stain for 15~30 minutes, see below solutions for stock stain concentration and adjust to the volume used accordingly. The staining box should be covered.

**NOTE:** Ethidium bromide is a suspected carcinogen, and the necessary safety precautions should be undertaken.

2. De-stain the gel for 10~30 minutes in distilled water again ensuring the gel is completely immersed.
3. Rinse the gel twice for a couple of seconds with distilled water.
4. Put the gel in a UV Transilluminator.

5. The samples will often appear as brighter, clearer bands when photographed or viewed using a gel documentation system. However, if the gel bands are too faint then the staining procedure should be adjusted so that there is less de-staining. If there is too much background, then the staining procedure should be adjusted so that there is more de-staining.

**Solutions:**

**1x TAE** 40mM tris (pH 7.6), 20mM acetic acid, 1mM EDTA.

50x (1L) dissolve in 750ml distilled water:

242g tris base (FW=121)

57.1ml glacial acetic acid

100ml 0.5M EDTA (pH 8.0)

Fill to 1 liter with distilled water.

**1x TBE** 89mM tris (pH 7.6), 89mM boric acid, 2mM EDTA.

10x (1L) dissolve in 750ml distilled water:

108g tris base (FW=121)

55g boric acid (FW=61.8)

40ml 0.5M EDTA (pH 8.0)

Fill to 1 liter with distilled water.

**Sample loading dye**

10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x TAE buffer. Only 1-10ml of the 10x loading dye should be prepared.

**Ethidium Bromide solution**

Add 10mg of Ethidium Bromide to 1ml distilled water.