Cleaver Scientific Ltd

Instruction Manual

Multi Sub Electrophoresis Systems

MSMAXI

MSSCREEN

Cleaver Scientific Ltd

Contents:-

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.

THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES:
73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992
EN 61010-1:1993/BS EN 61010-1:1993

MSMAXI10, MSMAXI15, MSMAXI20, MSMAXIDUO, MSMAXI25

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Tray Dams	Combs	Loading Guides	Cables
MSMAXI10	MS20-UV10 20 x 10cm (W x L)	MS20-UVDAM Pack of 2	2 x MS20-20-1 1mm, 20 sample	MS20-LG ï Strips MS20-WP ï Platform	CSL-CAB
MSMAX15	MS20-UV15 20 x 15cm (W x L)	MS20-UVDAM Pack of 2	2 x MS20-20-1 1mm, 20 sample	MS20-LG ï Strips MS20-WP ï Platform	CSL-CAB
MSMAXI20	MS20-UV20 20 x 20cm (W x L)	MS20-UVDAM Pack of 2	2 x MS20-20-1 1mm, 20 sample	MS20-LG ï Strips MS20-WP ï Platform	CSL-CAB
MSMAXIDUO	MS20-UV10 MS20-UV20	MS20-UVDAM Pack of 2	2 x MS20-20-1 1mm, 20 sample	MS20-LG ï Strips MS20-WP ï Platform	CSL-CAB
MSMAXI25	MS20-UV25 20 x 25cm (W x L)	MS20-UVDAM Pack of 2	2 x MS20-20-1 1mm, 20 sample	MS20-LG ï Strips MS20-WP ï Platform	CSL-CAB

MSSCREEN16, MSSCREEN24, MSSCREEN32, MSSCREENTRIO

Units include tank, lid and electrodes and include the following accessories:-

	Tray	Combs	Loading Guides	Cables
MSSCREEN16	MS26-UV16 26 x 16cm (W x L)	6 x MS26-28MC-1 1mm, 28 sample	MS26-LG ï Strips MS26-WP ï Platform	CSL-CAB
MSSCREEN24	MS26-UV24 26 x 24cm (W x L)	6 x MS26-28MC-1 1mm, 28 sample	MS26-LG ï Strips MS26-WP ï Platform	CSL-CAB
MSSCREEN32	MS26-UV32 26 x 32cm (W x L)	6 x MS26-28MC-1 1mm, 28 sample	MS26-LG ï Strips MS26-WP ï Platform	CSL-CAB
MSSCREENTRIO	MS26-UV16 MS26-UV24 MS26-UV32	6 x MS26-28MC-1 1mm, 28 sample	MS26-LG ï Strips MS26-WP ï Platform	CSL-CAB

Usage Guidance and restrictions:

ÅMaximum altitude 2,000m.

ÅTemperature range between 4°C and 65°C.

ÅMaximum relative humidity 80% for temperatures up to 31^{O}C decreasing linearly to 50% relative humidity at 40^{O}C.

ÅNot for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664.

POLLUTION DEGREE 2, states that: ñNormally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expectedò.

Care and Maintenance:-

Cleaning Horizontal Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60^{0} C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build up 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 to in detergents for more than 30 minutes.

The units 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 Alkalis.

Rnase Decontamination

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

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

RNaseZAPÊ (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

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 so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Instructions for fitting Loading Guides

These can be fitted to enhance visibility of the wells if desired. They can be fitted to the white vinyl platform sheet or to the unit itself.

1. Seat the tray in the unit and note the position of the comb grooves. The samples run black to red but the trays can be used frontward or backwards so ensure that the comb grooves closest to the black electrode are marked.
2. Remove the tray.
3. Peel the back off of the loading guide and carefully apply the loading guide directly to the gel platform.

The unit is now ready to be used.

Gel Preparation:-

Table 1 below shows the volume of agarose solution required to make the desired agarose gel for each unit tray size. For a standard 0.7% agarose gel, add 0.7 grammes of agarose to 100 ml of 1x TAE or TBE solution. The same 1 x solution should be used in the tank buffer solution.

Multi Sub Maxi	**Multi Sub Screen**		
Tray	**Gel volume for a 5mm thick gel**	**Tray**	**Gel volume for a 5mm thick gel**
20x10cm	100ml	**26 x 16cm**	**208ml**
20x15cm	150ml	**26 x 24cm**	**312ml**
20x20cm	200ml	**26 x 32cm**	**416ml**
250ml			

2. Add the agarose powder to a conical flask.

3. Add the appropriate amount of 1x TAE or TBE solution from the table above. To prevent evaporation during the dissolving steps below, the conical flask should be covered with parafilm.

4. Dissolve the agarose powder 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 around a 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:-

The CSL Multi sub range of units allows three different methods of gel casting:-

Casting Dams

Flexicaster

Traditional Tape

Using trays with Casting Dams:-

Fit the casting dams over each end of the tray and place onto a level surface. The dams should be fitted so that there is no gap between the sides of the tray and the groove in the dams. This will ensure that there is no possibility of gel leakage.

1. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
2. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
3. Allow the agarose to set, ensuring that the gel remains undisturbed.
4. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Using The Flexicaster: -

1. Level the Flexicaster base by adjusting the feet so that the bubble is exactly central.
2. Insert the desired length tray into the Flexicaster such that one end of the tray is pushed up and seals against the silicone mat of the permanent end of the Flexicaster.
3. Position the movable end of the Flexicaster so that the silicone mat is pushed against the other end of the tray.
4. Turn the cam so that the silicone mat tightly seals against the side of the tray. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
5. Allow the agarose to set, ensuring that the gel remains undisturbed.

6. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Using Traditional tape method:-

1. Autoclave or plastic backed general tape should be used. A length 5cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray and stuck m1cm in from the tray edge. This should then be folded and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Running the Gel:-

1. Mix the sample to be loaded with sample buffer ï see solutions for common sample buffers. Usually 3ul of sample buffer is adequate but less may be used with sample volumes of less than 10ul.

2. Fill the unit with buffer until the gel is just flooded with buffer. This will give the fastest resolution times. For enhanced quality of resolution of sample, fill the unit to 5mm above the gel.

3. Load the samples into the wells using pipettes. Multi-channel pipettes can be used for loading samples with MC compatible combs, see listing in accessories for identification of these.

4. Carefully place the lid on the tank and connect to a power supply (Not CSL-HBSET, slide the base section into the lid section, unit will run automatically.).
5. Typically gels are run at between 90 and 150 volts. However, maximum voltages are indicated on the serial badge of each unit. It should be noted that higher voltages generally give faster but poorer quality sample resolution.

Gel Staining and Viewing:-

The Multi Sub trays and the Mini Fast unit allow staining to be performed without removing the gel from the tray (unit for Fast Mini) if this is preferred.
1. Transfer the gel to a vessel containing the appropriate volume of 0.5 µg/ml ethidium bromide stain for 15ï 30 minutes, see solutions for stock stain concentration and adjust to the volume used accordingly. The entire gel should be covered.
NOTE:- Ethidium bromide is a suspected carcinogen and the necessary safety precautions should be undertaken.
2. De-stain the gel for 10ï 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. Transfer the gel to 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.

References

1. Sambrook, Fritsch, and Maniatis, **Molecular Cloning A Laboratory Manual,** Second Edition,
Cold Spring Harbor Laboratory Press, 1989.

2. **Current Protocols in Molecular Biology,** Greene Publishing Associates and Wiley-Interscience,1989.

Solutions:-

1x TAE 40 mM tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA.
50x (1L) dissolve in 750 ml distilled water:
242 g tris base (FW = 121)
57.1 ml glacial acetic acid
100 ml 0.5 M EDTA (pH 8.0).
Fill to 1 litre with distilled water.

1x TBE 89 mM tris (pH 7.6), 89 mM boric acid, 2 mM EDTA
10x (1L) dissolve in 750 ml distilled water:
108 g tris base (FW = 121)
55 g boric acid (FW = 61.8)
40 ml 0.5 M EDTA (pH 8.0)
Fill to 1 litre 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 1ï 10 ml of the 10x loading dye should be prepared.

Ethidium Bromide Solution

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

Combs – MC Denotes Multi Channel Pipette compatible.

Multi Sub Maxi	Multi Sub Maxi	Multi Sub Maxi	Multi Sub Maxi				
No. of Samples	**Part Number**	**No. of Samples**	**No. of Samples**	**No. of Samples**	**Part Number**	**No. of Samples**	**Part Number**
0.75mm	**Thick Comb**	**1mm**	**Thick Comb**	**1.5mm**	**Thick Comb**	**1.75mm**	**Thick Comb**
1	MS20-1-0.75	**1**	MS20-1-1	**1**	MS20-1-1.5	**1**	MS20-1-2
2	MS20-2-0.75	**2**	MS20-2-1	**2**	MS20-2-1.5	**2**	MS20-2-2
4	MS20-4-0.75	**4**	MS20-4-1	**4**	MS20-4-1.5	**4**	MS20-4-2
10	MS20-10-0.75	**10**	MS20-10-1	**10**	MS20-10-1.5	**10**	MS20-10-2
16	MS20-16-0.75	**16**	MS20-16-1	**16**	MS20-16-1.5	**16**	MS20-16-2
20	MS20-20MC-0.75	**20**	MS20-20MC-1	**20**	MS20-20MC-1.5	**20**	MS20-20MC-
25	MS20-25-0.75	**25**	MS20-25-1	**25**	MS20-25-1.5	**25**	MS20-25-2
30	MS20-30-0.75	**30**	MS20-30-1	**30**	MS20-30-1.5	**30**	MS20-30-2
36	MS20-36-0.75	**36**	MS20-36-1	**36**	MS20-36-1.5	**36**	MS20-36-2
40	MS20-40MC-0.75	**40**	MS20-40MC-1	**40**	MS20-40MC-1.5	**40**	MS20-40MC-

Multi Sub Screen Combs

Description	Part Number
Comb 28 sample MC, 0.75mm thick	MS26-28MC-0.75
Comb 56 sample MC, 0.75mm thick	MS26-56MC-0.75
Comb 28 sample MC, 1mm thick	MS26-28MC-1
Comb 56 sample MC, 1mm thick	MS26-56MC-1
Comb 28 sample MC, 1.5mm thick	MS26-28MC-1.5
Comb 56 sample MC, 1.5mm thick	MS26-56MC-1.5

Comb 28 sample MC, 2mm thick	MS26-28MC-2
Comb 56 sample MC, 2mm thick	MS26-56MC-2

Notes

Warranty

The Cleaver Scientific Ltd. (CSL) Multi Sub Horizontal Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, CSL will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than CSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by CSL or its associated distributors have invalidated warranty.

CSL cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

If a problem does occur then please contact your supplier or CSL on:-

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