Instruction Manual

Multi Sub Electrophoresis Systems

MSMIDI96

MSMIDI96ST

MULTISUB4

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

PACKING LISTS:

MSMIDI96

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

	Tray	Tray Dams	Combs and comb block	Loading Guides	Cables
MSMIDI96	MS10-UV96 10 x 12cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96-8-1-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI961.5	MS10-UV96 10 x 12cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96-8-1.5-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI96/2M	MS10-UV96 10 x 12cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96-8-1/2M-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI96/1.5/2M	MS10-UV96 10 x 12cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96-8-1.5/2M-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB

MSMIDI96ST

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

	Tray	Tray Dams	Combs and comb block	Loading Guides	Cables
MSMIDI96ST	MS10-UV96ST 10 x 24cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96ST-8-1-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI96ST1.5	MS10-UV96ST 10 x 24cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96ST-8-1.5-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI96ST/2M	MS10-UV96ST 10 x 24cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96ST-8-1/2M-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB
MSMIDI96ST/1.5/2M	MS10-UV96ST 10 x 24cm (W x L)	MS10-UVDAM Pack of 2	MSMIDI96ST-8-1.5/2M-CB	MS10-LG – Strips MS10-WP – Platform	CSL-CAB

MultiSUB4

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

	Trays	Combs	Caster	Cables
CSL-MULTISUB4	4 x MSUB4UV18 8 x 18cm (W x L)	8 x MSUB4-18/8-1.5 1.5mm, 18 and 8 sample	-	CSL-CAB
CSL-MULTISUB4EXCAS	4 x MSUB4UV18 8 x 18cm (W x L)	8 x MSUB4-18/8-1.5 1.5mm, 18 and 8 sample	1 x MSUB4EXCASTER External Caster For Msub4 (4 Trays)	CSL-CAB

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Please contact your supplier if there are any problems or missing items.

Usage Guidance and restrictions:

- Maximum altitude 2,000m.
- Temperature range between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31^{O}C decreasing linearly to 50% relative humidity at 40^{O}C.
- Not for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: “Normally only non-conductive pollution occurs.
Occasionally, however, a temporary conductivity caused by condensation must be expected”.

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.

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

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:-

1. 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 Midi 96	**Multi Sub Midi 96 Stretch**	**Multi Sub-4**	
Gel volume for a 5mm thick gel	**Gel volume for a 5mm thick gel**	**Tray**	**Gel volume for a 4x 5mm thick gels**
60ml	**120ml**	**8 x 6cm**	**96ml**
		8 x 12cm	**192ml**
		8 x 18cm	**288ml**

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 (Note for MultiSUB4):-

1. 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.
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.

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 1cm 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 – 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 enough buffer so that it will just cover the gels when they are immersed. This will give the fastest resolution times. For enhanced quality of resolution of sample, fill the unit to 5mm above the gel.
3. On the bench surface, load a small amount of running buffer to flood the wells. Load the samples into the wells using a pipette. Multi-channel pipettes can be used for loading samples into wells formed by MC compatible combs, see listing in accessories for identification of these.
4. Once loaded, gently immerse the gels within the buffer, stacking them carefully on top of each other. Replace lid and connect unit to the power supply using cables.
5. Typically gels are run at between 90 and 150 volts (50V MSUB4). 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 if this is preferred.

1. Transfer the gel to a vessel containing the appropriate volume of 0.5 µg/ml ethidium bromide stain for 15–30 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 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. 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 1–10 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 Midi 96 Combs

Description	Part Number
MSMIDI 96 Comb 8 sample MC + 1 Marker, 1mm thick COMB BLOCK	MSMIDI96-8-1-CB
MSMIDI 96 Comb 8 sample MC + 1 Marker, 1.5mm thick COMB BLOCK	MSMIDI96-8-1.5-CB
MSMIDI 96 Comb 8 sample MC + 2 Marker, 1mm thick COMB BLOCK	MSMIDI96-8-1/2M-CB
MSMIDI 96 Comb 8 sample MC + 2 Marker, 1.5mm thick COMB BLOCK	MSMIDI96-8-1.5/2M-CB
MSMIDI 96 Comb 8 sample MC, 1mm thick. one marker lane.	MSMIDI96-8-1
MSMIDI 96 Comb 8 sample MC, 1.5mm thick. one marker lane.	MSMIDI96-8-1.5
MSMIDI 96 Comb 8 sample MC, 1mm thick. two marker lanes.	MSMIDI96-8-1/2M
MSMIDI 96 Comb 8 sample MC, 1.5mm thick. two marker lanes.	MSMIDI96-8-1.5/2M

Multi Sub Midi 96 STRETCH Combs

Description	Part Number
MSMIDI 96 STRETCH Comb 8 sample MC + 1 Marker, 1mm thick COMB BLOCK	MSMIDI96ST-8-1-CB
MSMIDI 96 STRETCH Comb 8 sample MC + 1 Marker, 1.5mm thick COMB BLOCK	MSMIDI96ST-8-1.5-CB
MSMIDI 96 STRETCH Comb 8 sample MC + 2 Marker, 1mm thick COMB BLOCK	MSMIDI96ST-8-1/2M-CB
MSMIDI 96 STRETCH Comb 8 sample MC + 2 Marker, 1.5mm thick COMB BLOCK	MSMIDI96ST-8-1.5/2M-CB
MSMIDI 96 Comb 8 sample MC, 1mm thick. one marker lane.	MSMIDI96-8-1
MSMIDI 96 Comb 8 sample MC, 1.5mm thick. one marker lane.	MSMIDI96-8-1.5
MSMIDI 96 Comb 8 sample MC, 1mm thick. two marker lanes.	MSMIDI96-8-1/2M
MSMIDI 96 Comb 8 sample MC, 1.5mm thick. two marker lanes.	MSMIDI96-8-1.5/2M

MultiSUB 4 Combs:-

Description	Part Number
12/1 SAMPLE 1.5mm COMBS FOR multiSUB4	MSUB4-12/1-1.5
18/8 SAMPLE 1.5mm COMBS FOR multiSUB4	MSUB4-18/8-1.5

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 it's 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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