LFVERTICAL

Instruction Manual

Large Format

Vertical

Electrophoresis Systems

Catalogue Numbers

CSQ20

CSQ33

V03.03.15

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.
ACRYLAMIDE IS A POWERFUL NEUROTOXIN IN SOLUTION FORM.
POLYMERIZED GELS CAN CONTAIN SOME UNPOLYMERIZED SOLUTION AND PROTECTIVE GLOVES AND CLOTHING MUST BE WORN.
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

CSQ20, CSQ33

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

	Glass Plates	Comb	Spacers	Cables
CSQ20	NOTCHED, 1 PLAIN, 1	CSQ20-0.35-48	CSQ20-S0.35	CSL-CAB2
CSQ33	NOTCHED, 1 PLAIN, 1	CSQ33-0.35-48	CSQ33-S0.35	CSL-CAB2

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.

Care and Maintenance

Cleaning Large Format Vertical 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.

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”.

Setting up the Large Format 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.

The unit is now ready to be used.

Cleaning and Preparation of Glass Plates

Better and more consistent results will be obtained if care is taken to ensure that the glass plates are as clean as possible. New glass plates must be cleaned in the same way as used glass plates because these will contain surface debris that may interfere with the gel.

First, clean using a neutral detergent and a small brush.

Do not use metal wool or other test tube brushes, abrasive cleaning creams or scourers because these can scratch the surface of the glass plates.

The glass plates should be washed in the following sequence – Distilled water ethanol, acetone, ethanol, distilled water. Thoroughly rinse and dry the glass plates before use. For extra clean plates, these should be wiped with a microscope tissue soaked in chloroform or dichloroethane in a fume hood.

To ease separation of the gel from the glass plates once the gel has been run, it is advisable to siliconise the notched glass plate with a tissue soaked in Dimethyldichlorosilane. Wipe the plate, including the ears, in a fume hood. Rinse with water and dry with a tissue.

The plain glass plate should be siliconised along the outer 1cm lengths where the spacers will be positioned.

This should be periodically repeated when the gels start to stick to the plates. Plates should then be cleaned and siliconised as described above.

The horizontal gel pouring method described in this email will not work if both plates are siliconised. In that case, use an alternative gel pouring method or do not siliconise the plain glass plate as described above.

The above procedures are not necessary every time a gel is poured.

After use, first, clean using a neutral detergent and a small brush and wahs with distilled water, ethanol and acetone as described above.

DO NOT ALLOW ORGANIC SOLVENTS INCLUDING ACETONE AND ALCOHOLS TO COME INTO CONTACT WITH THE PLASTIC COMPONENTS OF THE MAIN UNIT.

Reagent Preparation and Gel Volumes

For consistent gels it is advisable to use high quality reagents and where possible deionisine, degass and filtrate acrylamide gel solutions prior to use.

Made up Acrylamide solutions should be stored in a refrigerator and allowed to reach room temperature prior to pouring.

It is always advisable to work using stock solutions which allow added convenience and save time when it comes to gel pouring. Pages 16 to 17 list stock solutions for SDS PAGE gels which should be pre-made beforehand. For DNA Sequencing see page 18. For native gel formulae and running conditions, please consult a laboratory manual.

As a guide, polymerisation conditions should be adjusted to effect polymerisation within about 5 - 15 minutes. Test a small volume in a vial prior to pouring the gel. As a rough guide 100ml of degassed 6% acrylamide gel will set in about 5 minutes at room temperature when gently mixed with 450□l of freshly prepared 10% (w/v) Ammonium persulphate and 200□l TEMED. The setting time increases to about 10 minutes if the TEMED volume is reduced to 100□l and to approximately 15 minutes with 75□l.

The amount of catalysts may need to be reduced under warm conditions.

Gel Volumes:

Table 1 below shows the total volume of gel solution required.

Table 1.

CSQ20		CSQ33	
	Total Gel volume for a 1mm thick gel.		Total Gel volume for a 1mm thick gel.
For different thicknesses of gel, multiple the below amounts by the spacer thickness.			
Single – one gel	80ml	Single – one gel	125ml

Gel Selection:-

For protein gels, care should be taken when selecting the pore size of the gel to be used. The pore size or % of gel determines the resolving ability given different sizes of protein.

See Table 2 below which details which percentage of gel to use to separate the sizes of proteins indicated.

Table 2.

Acrylamide Percentage	Separating Resolution
5 %	60 - 220 KD
7.5 %	30 - 120 KD
10 %	20 - 75 KD
12%	17 – 65 KD
15 %	15 -45 KD
17.5%	12 – 30 KD

Gel Pouring

For Gel Thicknesses 0.25 and 0.35mm, follow the instructions below. For Gel Thicknesses thicker than 0.35mm, first securely tape the bottom of the gel with electrical tape then follow the instructions below.

1. Lay the plain glass plate on a flat surface and arrange the spacers perfectly aligned with the edges of the plate.
2. Carefully place the notched glass plate on top of the plain glass plate and clamp the plates together using bulldog clips arranged along the edge of the glass plates, the pressure clamps of these should be in line with the spacers.
3. Fill a syringe with the required gel mix, see Table 1. for gel volume required and pages 16 to 18 for gel solutions. Be careful not to agitate or to introduce bubbles into the solution.
4. Position the syringe above one edge of the notch in a vertical position. Steadily eject the gel solution along the notched area moving the syringe spout smoothly from one side of the notch to the other. The gel mix should form a continuous pool along the top of the gel space move down between the glass plates.
5. Be careful not to overfill the notched area, fill gradually – the gel solution should be around half the height of the notches on the notched glass plate. Also ensure not to under fill the notched area as air bubbles are more likely to be introduced between the glass plates. The boundary of the gel should migrate as a straight line. To prevent or expel bubbles, the glass plates can be tapped lightly behind the moving gel boundary to prevent any bubble formation.
6. When the gel boundary reaches the bottom of the glass plates, remove all the surplus gel from the notched area with the syringe. This will ensure that the gel mix doesn't drip from the bottom of the glass plates.
7. Insert the comb. If a square well comb is used, insert the teeth making sure no bubbles are trapped. When using a shark's tooth comb, insert the flat face of the comb at a slight angle to prevent bubbles from being trapped. A few drops of gel mix can be added if necessary.

8. Carefully straighten the shark's tooth comb so that it is parallel to the top of the gel plate and reaches 3 - 5mm below the notched area.
9. For low percentage and DNA sequencing gels, leave to polymerise completely for at least 90 minutes. Low percentage gels can be left to polymerise overnight. To prevent the ends of the gel from drying out use wet tissues under a nesco film seal.

Assembling the Unit

Insert the lower buffer chamber into position.
Remove the bulldog clips and the bottom tape, if used, from the glass plates.
Insert the glass plates behind the clamping bars and tighten the screws.
DO NOT OVER-TIGHTEN THE GEL PLATE CLAMPING SCREWS as this may lead to the glass plate breakage and will also make the insertion and removal of combs difficult.
Attach the electrical connectors to the buffer chambers.

Run Conditions and Buffer Volumes

Ensure that the buffer drainage tap is in the closed position.

CSQ20

Into the upper buffer chamber pour between 250ml min and 500ml max of electrophoresis running buffer.
Into the bottom buffer chamber pour between 250ml min and 500ml max of electrophoresis running buffer.
IMPORTANT do not fill over the Maximum fill lines.

CSQ33

Into the upper buffer chamber pour between 400ml min and 1000ml max of electrophoresis running buffer.
Into the bottom buffer chamber pour between 400ml min and 1000ml max of electrophoresis running buffer.
IMPORTANT do not fill over the Maximum fill lines.

Prior to loading samples, flush out the wells with running buffer to clear them of urea and debris.

Preparation of DNA Sequencing samples for loading:

1. The volume of sample depends on the capacity of the wells (See Comb specifications page).
2. Heat the sequencing samples in a water bath or heating block at 95^{0}C for 3 minutes, place on ice and centrifuge - 12,000xg for 3 minutes. Return to ice.

Preparation of denatured protein samples for loading:

The instructions given below are for denatured samples. For Native samples, please consult a laboratory handbook.

1. Prepare the protein samples for loading. The volume of sample depends on the capacity of the wells (See Comb specifications page).
2. Using a 0.5 ml micro-centrifuge tube or other convenient receptacle, combine the protein sample and 4 X sample buffer. It is always advisable to use protein markers in one of the end lanes to indicate sizes of bands. These should be prepared according to the manufacturers instructions.
3. Heat the samples in a water bath or heating block for 2 minutes to denature the samples.
4. Centrifuge the samples in a micro-centrifuge for 20 seconds at 12,000 rpm. The protein samples are now ready to load.

Gel Running:

1. Remove the Square tooth comb before loading the samples. If using a Shark's tooth comb, leave this in position as the comb teeth will act as the wells.
2. Load the required volume of sample using a suitable loading tip; consult the comb specifications page 19 for details on maximum sample volume for the comb and gel thickness used. If possible avoid taking the sample from the bottom of the tube - particulate materials may cause streaking or smearing.
   Sample dispersion can be minimized by loading the sample directly onto the bottom of the well and keep it as a thin layer.
3. Fit the safety lid ensuring it is positioned fully down over the electrical connectors.
4. Connect and run the gel at the desired power setting. The leads and electrical connectors are CE safe to 1,500 Volts and users are advised not to exceed this voltage.
5. Typically for DNA Sequencing gels, 45 -55 Watts constant power is advised. For other types of gel please consult a laboratory handbook.
6. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4Kd in size.

Ending the Run

Disconnect and turn off the power supply before removing the connectors.

Remove the safety lid by gripping the edges of the lid and pushing down with your thumbs on the pegs located on the top of the unit.

Separate the plates with a strong, thin, broad blade. Do not force the glass plates apart at the notch as this may damage the plates. The gel will usually stick to one of the plates and can be removed by first soaking in buffer and then gently lifting with a spatula.

For protein gels, the gel is now ready to be stained with Coomassie or silver stain or the proteins in the gel can be transferred to a membrane by electroblotting for specific band identification and further analysis.

For DNA Sequencing gels, the gel should be attached to filter paper and dried. Please consult a laboratory handbook for the further handling of Sequencing gels.

Disconnect the leads to the power supply and remove the lid. Then disconnect the lead to the lower buffer chamber.

Remove the bottom tank and empty then position the bottom tank around the back of the unit underneath the drainage tap. Open the valve and the buffer will then flow into the bottom buffer tank.

Again empty the lower buffer tank and carefully discard the buffer, this cannot be re-used.

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.

Stock Solutions for SDS PAGE gels:-

Stock 30% Acrylamide Gel Solution:-

30.0 g acrylamide

0.8 g methylene bisacrylamide

Distilled Water to 100ml

Stock 4 X Resolving Gel Tris (1.5 M Tris HCl pH8.8, 0.4 % SDS)

To 110ml Distilled Water add 36.4 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 8.8 with 1N HCl

Adjust the final volume to 200ml with Distilled Water.

Stock 4 X Stacking Tris (0.5 M Tris HCL pH6.8, 0.4 % SDS)

To 110ml Distilled Water add 12.12 g of Tris base

Add 8ml of 10 % SDS

Adjust pH to 6.8 with 1N HCl

Stock 4 X Tris-glycine tank buffer - SDS

36 g Tris base

172.8 g glycine

Distilled Water to 3 L

1 x Tris-glycine tank buffer - SDS

750ml of 4 X Tris-glycine reservoir buffer - SDS

30ml of 10 % SDS

Distilled Water to 3L

Add Distilled Water to a final volume of 200ml

10 % AP (ammonium persulphate solution)

0.1 g ammonium persulphate

1ml Distilled Water

TEMED

Stock 4 X Sample Buffer

4ml glycerol

2ml 2-mercaptoethanol

1.2 g SDS

5ml 4 X Upper Tris

0.03 g Bromophenol blue

Aliquot into 1.5ml microcentrifuge tubes. Store at -20°C.

Stock Solutions for DNA Sequencing gels:-

10 x DNA Sequencing Buffer

164.0 g Tris-OH

27.5 g Boric Acid

7.45 g disodium EDTA

Add Distilled Water to a final volume of 1L

Acrylamide stock

38% Acrylamide

2% bis-acrylamide

8% gel CSQ33 Gel

40.4 g urea

27.0 ml water

16.8 ml 38/2 acrylamide

8.0 ml 10 x DNA Sequencing Buffer

6% CSQ33 Gel

40.4 g urea

31.2 ml water

12.6 ml 38/2 acrylamide

8.0 ml 10 x DNA Sequencing Buffer

5% CSQ33 Gel

40.4 g urea

33.5 ml water

10.5 ml 38/2 acrylamide

8.0 ml 10 x DNA Sequencing Buffer

If necessary mix by heating slightly, degassing is advised. Add 0.7 ml 10% ammonium persulphate, and 25ul TEMED, and pour gel immediately.

Combs:–

CSQ20:-

Code	Description	Sample Volume µl for a 5mm thick gel
CSQ20-0.25-24	Comb 24 sample, 0.25mm thick, Sharks tooth	7
CSQ20-0.25-48	Comb 48 sample, 0.25mm thick, Sharks tooth	3
CSQ20-0.35-24	Comb 24 sample, 0.35mm thick, Sharks tooth	9
CSQ20-0.35-48	Comb 48 sample, 0.35mm thick, Sharks tooth	5
CSQ20-1-24	Comb 24 sample, 1mm thick, Square tooth	40
CSQ20-1-48	Comb 48 sample, 1mm thick, Square tooth	20
CSQ20-1.5-24	Comb 24 sample, 1.5mm thick, Square tooth	60
CSQ20-1.5-48	Comb 48 sample, 1.5mm thick, Square tooth	30

CSQ33:-

Code	Description	Sample Volume µl for a 5mm thick gel
CSQ33-0.25-48	Comb 48 sample, 0.25mm thick, Sharks tooth	7
CSQ33-0.25-96	Comb 96 sample, 0.25mm thick, Sharks tooth	3
CSQ33-0.35-48	Comb 48 sample, 0.35mm thick, Sharks tooth	9
CSQ33-0.35-96	Comb 96 sample, 0.35mm thick, Sharks tooth	5
CSQ33-1-48	Comb 48 sample, 1mm thick, Square tooth	35
CSQ33-1-80	Comb 80 sample, 1mm thick, Square tooth	20
CSQ33-1.5-48	Comb 48 sample, 1.5mm thick, Square tooth	50
CSQ33-1.5-80	Comb 80 sample, 1.5mm thick, Square tooth	30

Warranty

The Large Format Vertical 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, your supplier 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 your supplier 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 your supplier or it's associated distributors have invalidated warranty.

Your supplier 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.