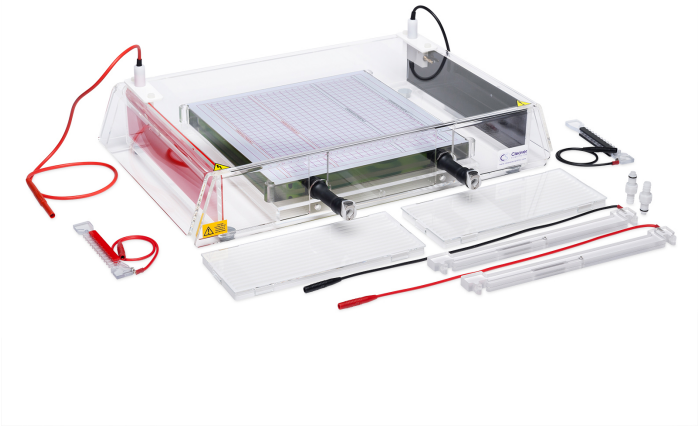
IEF System



Flatbed Isoelectric Focusing Unit

Instruction Manual

Catalogue Numbers

CSL-IEF

Record the following for your records:

Model _____

Catalogue No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

Contents

Safety Information

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 following European directives:

2014/35/EU Low Voltage Directive and 2014/30/UE (official Title 2004/108/EC) EMC Electromagnetic Compatibility

By virtue of the following harmonised standards:

BS EN IEC 61010-1: 2010 Safety Testing of Lab Equipment

BS EN IEC 61326-1:2013 EMC Electro Magnetic Compatibility

ROHS DIRECTIVE 2011/65/EU

BS EN 50581:2012 Restriction of Hazardous Substances

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".

Packing List

No. of items	Description
1	Main Unit ❖ Base ❖ Lid
1	Cooling Plate
2	Cathode Electrodes
2	Anode Electrodes
1	Glass Electrode Frame
2	Power Cables
1	IPG Strip Focusing Tray
1	IPG Rehydration Tray
1	Instruction Manual

Packing List Checked by: _____

Date: _____

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.

Cleaver Scientific is liable for all missing or damaged parts / accessories within 7 days after customers have received this instrument package. Please contact Cleaver Scientific immediately regarding this issue. If no response within such period is received from the customer, Cleaver Scientific will no longer be liable for replacement/damaged parts.

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

Specifications

Capacity	1 to 12 IPG Strips IEF gel	7 to 24cm long Up to 24 cm x 22 cm
Platform dimension	25 cm x 23 cm	
Operating Conditions	Maximum Voltage Maximum Current Power	3000 V 300mA 300W
Platform Temperature	7 to 20 ◦C	
Dimensions (LxWxD)	58 cm X 45 cm X 13 cm	
Weight	8.5 kg	
Environmental Operating Conditions	Maximum Altitude Temperature Range Humidity Not for outdoor Use	2,000 m 4°C - 65°C Up to 80%

Care and Maintenance

Cleaning CSL Isoelectric focusing Units

The unit 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 plate electrodes and vigorous cleaning is not necessary or advised. Air drying is preferable before use.

Units are best cleaned using warm water and a mild concentration of soap or other mild detergent.

- Water at temperatures above 60^0 C can cause damage to the unit and components.
- Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons.

  Note: The unit should not be left in detergents for more than 30 minutes.)
- The units should never come into contact with the cleaning agents like Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol, Alkalis. These will cause irreversible and accumulative damage.

RNase Decontamination

RNase Decontamination can be performed using the following protocol:- Clean the unit with a mild detergent as described above. Then wash with 3% hydrogen peroxide (H2O2) for 10 minutes and finally 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.)

Operating the IEF System

Setting up the Cooling Plate

The unit is provided with a cooling plate which acts a platform for running the IPG strips and prevents overheating of the strips when attached to a recirculating chiller. The cooling plate consists of two sealing ports which when removed will seal the cooling plate. These should be removed before connecting to the chiller by pressing down on the metal button at the top of each port.

- Connecting the chiller to the cooling plate:-

Connect the tubing from the chiller to both port sealers. Insert the port sealers onto the ports; this will now render these in the open position.

Note: The chiller must be set between 16-17 °C for running IPG Strips and between 2-4 °C for running the Precast Gels for Isoelectric Focusing. Switch on the chiller at least 10 minutes prior to electrophoresis to allow the cooling plate to reach the desired temperature.

Running IPG Strips

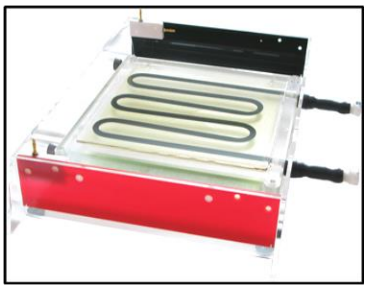
IPG Strip Sample Rehydration

1. Lay individual IPG strips within the rehydration channels in the CSL-RHDTRAY
2. Incubate IPG strips overnight with buffer (8M Urea, 1% CHAPS, 13mM DTT and 0.5% SERVALYT™ 3-10, corresponding to the pH gradient of the IPG strip) containing SERVA proteome markers. At least 5 and 50µg of protein is sufficient for 7 and 18cm IPG strips respectively.

3. Overlay each strip with silicone oil to prevent desiccation during the overnight incubation period.

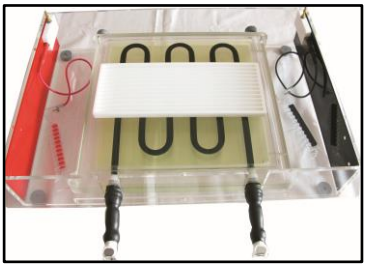
1. Place the unit on the bench in a convenient position.

2. Insert the cooling plate in the base unit. Ensure it locates properly with the pipes protruding from the cuts in the side wall. Set the desired temperature of the chiller and switch it on at least 10 minutes prior to electrophoresis to allow the cooling plate to reach the desired temperature.



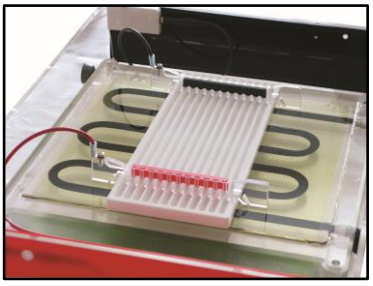
3. Connect each adjustable electrode to the gold plugs fixed within the tank.
The electrodes are colour coded for correct polarity orientation.

4. Place the IPG strip focusing tray directly onto the cooling plate.

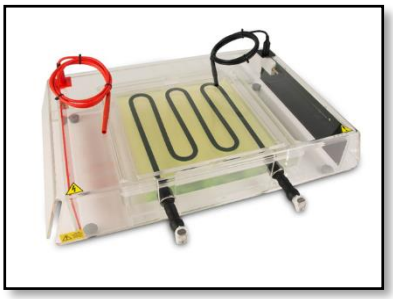


5. Using a forceps place the IPG strips into the running tray with the gel side facing upwards. Then cover the anode and cathode ends of the IPG strips with electrode wicks saturated with deionised water. Cover the IPG strips with enough Silicone oil before starting the run.

6. Gently clip both the electrodes over the anodic and cathodic ends of the IPG strip respectively.



7. Screw the power cables into the lid before placing it on top of the gel tank. Then connect the power cables to the power supply and the unit is ready for Isoelectric Focusing.



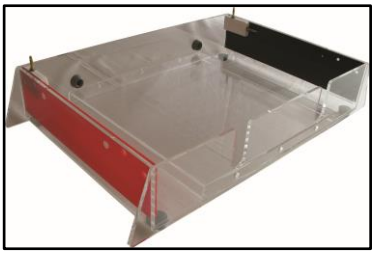
Recommended running conditions for IPG strips

Recommended Running Conditions for IEF of 7cm SERVA IPG strips	*Voltage Step*	*1*	*2*	*3*	*4*	*5*	*6 end of run*
	Voltage (V)	*150*	*300*	*600*	*1500*	*3000*	*330*
	Time (h)	*0.5*	*0.5*	*0.5*	*0.5*	*2.5*	*<20*
	Volt-hours	*75*	*150*	*300*	*750*	*7500*	*-*
Recommended Running Conditions for IEF of 18cm SERVA IPG strips	*Voltage Step*	*1*	*2*	*3*	*4*	*5 end of run*	
	Voltage (V)	*300*	*600*	*1500*	*3000*	*330*	
	Time (h)	*1*	*1*	*1*	*12.5*	*<20*	
	Volt-hours	*300*	*600*	*1500*	*37500*	*-*	

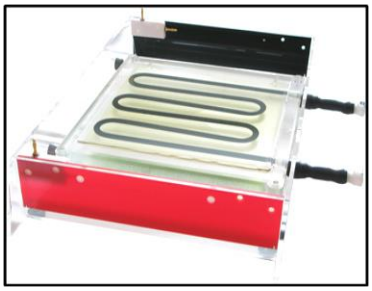
Installation and Running Precast or Hand-cast Gels

The composition and running of IEF gels varies considerably depending on the range of pH required, the application (native or denaturing) and the format of the gels. The following instructions are general instructions for Isoelectric focusing applications where the gels are hand poured or precast in a horizontal format on a gel support (not supplied). Please consult a suitable reference text for gel compositions, recommended temperatures, voltage and run times.

1. Place the unit on the bench in a convenient position.



2. Insert the cooling plate in the base unit. Ensure it locates properly with the pipes protruding from the cuts in the side wall. Set the desired temperature of the chiller and switch it on at least 10 minutes prior to electrophoresis to allow the cooling plate to reach the desired temperature.



3. For running precast gels place the electrode frame (CSL-IEFFRME) directly above the cooling plate, ensuring that the pins within the frame are aligned with the holes of the cooling plate.

4. Apply 1-5ml of Triton X100 non-ionic detergent (or Kerosene) to the surface of the cooling plate. This will act as a heat transfer agent between the cooling plate and the gel, during the run. Place the gel on its support onto the Cooling plate in the position required, making contact with the applied Triton X100 on the lower edge. Slowly lower the gel so that the Triton spreads under the gel support plate, expelling any air and ensuring a good contact to the cooling plate surface. Remove all excess Triton from the cooling plate.

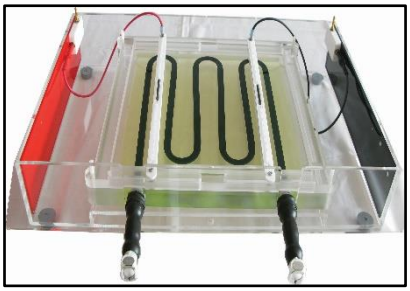
5. Prepare two electrode strips, one cathode, one anode, by cutting strips from filter paper which are slightly shorter than the gel width. Moisten the Electrode strips with the relevant electrode solutions and drain the strips if required.

6. Place the electrode strips 2mm from the cathode and anode edges of the gel. The etched positions will help in positioning. Ensure the electrode strips are slightly shorter than the gel on which they are applied. This will prevent electrical contact along the edges of the gel. If using a precast IEF gel samples may be loaded by pipettes into the wells of a sample applicator strips (not supplied) overlaid across the width of the gel at its centre. If using a precast agarose gel simply load the samples in the wells created by the comb.

7. Apply the samples and proceed with steps 9 to 13 if pre-focusing is not required. If prefocusing is required then proceed with steps 9 to 13 before loading samples and repeating these steps. Pre-focusing is generally performed at lower voltage than the following separation step.

8. Using the markings on the electrode holder which correspond to the positions of the electrodes on the cooling plate, align the electrodes in the correct position. This is achieved by loosening the screws on the electrode and sliding them within the channel on the plate holder and retightening the location screws.

9. Place the electrode into the unit and lower carefully so that the platinum wire of the electrodes makes contact with the filter paper wicks on the gel.



10. Connect the electrodes to their respective sockets in the base unit. Then place the safety lid and connect to a suitable power supply.

11. The unit is now ready to be switched on for the run.

End of Run

1. Once the run is complete turn of the power supply and the chiller. The chiller should be disconnected by pressing the metal button on the sealing ports. If these are not used, liquid from the chiller can leak from the chiller and cooling plate.
2. Unscrew the power cables and remove the safety lid.
3. Then carefully remove the electrode wicks without damaging the strips.
4. The IPG strips are now ready for fixation and staining, or SDS-PAGE for 2D electrophoresis.

IPG Strip Fixation and Staining

I. Cleaver Scientific recommends SERVA Blue R (35051) for protein staining according to the protocol laid out below.

II. Immerse the IPG strips in 100ml of fixing solution (40ml ethanol, 10ml glacial acetic acid and 50ml water), agitating gently for 30 minutes on a rocking platform (CSL3DSHAKER) set at 50 to 100rpm.

III. Mix together stock solution 1 (0.2% SERVA Blue R, 90% ethanol [11094]) and stock solution II (20% acetic acid) before adding the IPG strips and incubating for a further 20 minutes on the rocking platform.

IV. Begin destaining the IPG strips by rinsing them in fixing solution for 30 seconds.

V. Mix 20ml of ethanol with 10ml of glacial acetic acid and 70ml of distilled water, while the IPG strips are still in the fixing solution. Transfer the IPG strips to the destaining solution and incubate the IPG strips until discrete protein bands are visible upon a clear background.

VI. Rinse the IPG strips for 5 minutes in distilled water. Repeat. The IPG strips should now be ready for visualisation.

Strip preparation for second dimension SDS-PAGE

IPG strips are often used in the first dimension of 2D-electrophoresis, where proteins are initially resolved by their isoelectric point and, then, by their molecular weight, in the second dimension, using SDS-PAGE. However, before second dimension SDS-PAGE can take place the strips must be equilibrated after first-dimension IEF.

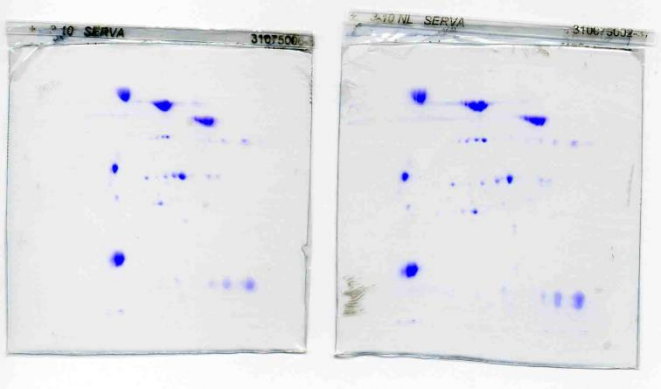
1. Transfer the IPG strips from the CSL-IEF unit to a clean rehydration tray (CSL-RHYDTRY). Using tweezers or forceps gently lower each strip, gel side upwards, into the bottom of each channel within the rehydration tray. Cover each 7 and 18cm strip respectively with 3 and 7ml of equilibration buffer I (50mM Tris-HCl, pH 8.8; 6M Urea; 30% glycerol; 2% SDS; 0.01% bromophenol blue and 1% (w/v) DTT). Incubate gently for 10 minutes on an agitating platform.

2. Carefully remove equilibration buffer I from rehydration tray, taking care to prevent the IPG strips from falling out of the tray. Add the same volumes of equilibration buffer II (50mM Tris-HCl, pH 8.8; 6M Urea; 30% glycerol; 2% SDS; 0.01% bromophenol blue and 5% iodoacetamide) to each channel, as described in step 1.

3. Incubate for 10 minutes.

4. Remove each IPG strip from the tray and dip it briefly in 1 x Laemmli buffer, before overlaying the strip with forceps along the top of a pre-made acrylamide gel, ensuring that there are no air bubbles between the strip and the gel. Please note that only the 1-mm thick edge of IPG strip should touch the edge of the gel otherwise resolution will be compromised.

5. Cover the strip with melted 0.5% (w/v) agarose to keep it in position on top of the gel. Once the agarose has set, the gel is then ready for second-dimension electrophoresis. Cleaver Scientific recommends the VS20WAVESYS and VS20WAVE-IEFKIT for use with 18cm IPG strips. For a 20 x 20cm gel format, Cleaver Scientific suggests an initial constant current setting of 20mA per gel. Please refer to the VS20WAVESYS manual for further information.



SERVA Proteome markers (39220.01) after 2-D electrophoresis using 18-cm long SERVA IPG Bluestrips (pH 3-10) on the CSL-IEF unit in the first dimension, followed by SDS-PAGE in the VS20WAVEDSYS unit in the second dimension.

General maintenance post IEF

1. After use, remove the electrodes from the glass electrode frame and rinse them carefully with distilled water to prevent corrosion by the strong acidic and basic solutions. DO NOT submerge the socket connector.

2. Rinse the buffer chambers with distilled water, again taking care not to submerge the socket connector. Dry the chambers by using a vacuum line avoiding damage to the platinum electrodes.

Warranty

The Cleaver Scientific Ltd. (CSL) Isoelectric focusing 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:-

Cleaver Scientific Ltd.
Unit 41
Somers Road Industrial Estate
Rugby
CV22 7DH
Tel: +44 (0)1788 565300
Fax: +44 (0)1788 552822
Email: info@cleaverscientific.com

Recommended Isoelectric Focusing and 2-D Electrophoresis Products & Spares

Isoelectric Focusing

CSL-IEF	Flatbed IEF system for IEF strips and gels
CSL-CHILLER	Chiller System, -20 - 100^{oC}, inc tubing & clips - 240V
CSL-CHILLER$	Chiller System, -20 - 100^{oC}, inc tubing & clips - 115V
CSL-IEF-KIT	CSL-IEF, CSL-CHILLER and EV3330
CSL-IEFPOS	Replacement positive electrode
CSL-IEFNEG	Replacement negative electrode
CSL-IEFPLT	Replacement Glass platform
EV3330	Consort 3000V, 300mA, 300W power supply
CSL-IEFFRME	Replacement electrode frame
CSL-RHYDTRY	REHYDRATION TRAYS
CSL-FOCUSTRAY	Focusing tray with adjustable electrodes

2-D packages

CSL-IEF-KIT-MINI	CSL-IEF-KIT, CVS10DSYS, VS10-IEFKIT
CSL-IEF-KIT-WAVE	CSL-IEF-KIT, VS20WAVESYS, VS20WAVE-IEFKIT
CSL-IEF-KIT-MAXIPLUS	CSL-IEF-KIT, VS30DSYS

IEF-CONVERSION KIT

VS10-IEFKIT	VS10PGS1/6, VS10-1-2D; IEF Conversion Kit for 7cm IPG strips and Tube gels
VS20WAVE-IEFKIT	VS20PGS1/6, VS20-1-2D; IEF Conversion Kit for 18cm IPG strips and Tube gels

Notes