Instruction Manual



omniPAGE

High Intensity Blotting Modules

Catalogue Numbers

VS10BI-HI, VS20BI-HI

Supplied with standalone blotting systems:

SB10-HI, SB20-HI

& with modular electroblotting systems:

CSV10CBS-HI, VS20CBS-HI

& with the complete modular system:

WAVECES-HI

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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.

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 following European directives:

2006/95/CE 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

PACKING LISTS:

High Intensity Blotting Modules

VS10BI-HI

10x10cm high intensity blotting insert, includes:

VS10BI-HI	Blotting Insert	Cassettes	Fibre pads	Cooling Pack	Cables
	1x omniPAGE 10x10cm High Intensity Blot insert (plate electrodes)	**2x SB10C**	**1x SB10F Pk/8**	-	-

Packed & Checked By:..... Date.....

VS20BI-HI

20x20cm high intensity blotting insert, includes:

VS20BI-HI	Blotting Insert	Cassettes	Fibre pads	Cooling Pack	Cables
	1x WAVE 20x20cm High Intensity Blot insert (plate electrodes)	**1x SB20C**	**1x SB20F Pk/6**	-	-

Packed & Checked By:..... Date.....

Standalone Blotting Systems

SB10-HI, 10x10cm

Units include tank and lid **plus** the following accessories:-

SB10-HI	Blotting Insert	Cassettes	Fibre pads	Cooling Pack	Cables
	As per VS10BI-HI			1x VS10CB	1x CSL-CAB

Packed & Checked By:..... Date.....

SB20-HI, 20x20cm

Units include tank and lid **plus** the following accessories:

SB20-HI	Blotting Insert	Cassettes	Fibre pads	Cooling Coil	Cooling Pack	Cables
	As per VS20BI-HI			1x VS20WAVE-CC	1x VS20CB	1x CSL-CAB

Packed & Checked By:..... Date.....

Modular Electroblotting Systems

CVS10CBS-HI, 10x10cm

Units include tank and lid **plus** the following accessories:

CVS10CBS-HI	PAGE Insert	Casting Base	Glass Plates	Combs	
	1x VS10DIRM	1x VS10CB	1x VS10PGS1 1x VS10NG	2x VS10-12-1	
	Blotting Insert	**Cassettes**	**Fibre Pads**	**Cooling Pack**	**Cables**
	As per VS10BI-HI			1x VS10CB	1x CSL-CAB

Packed & Checked By:..... Date.....

VS20CBS-HI, 20x20cm

Units include tank and lid **plus** the following accessories:

VS20CBS-HI	PAGE Insert	Casting Base	Glass Plates	Combs	Cooling Coil
	1x VS20WAVEDIRM	1x VS20WAVECAST	1x VS20PGS1 1x VS20NG	2x VS20-24-1	1x VS20WAVE-CC
	Blotting Insert	**Cassettes**	**Fibre Pads**	**Cooling Pack**	**Cables**
	As per VS20BI-HI			1x VS20CB	1x CSL-CAB

Packed & Checked By:..... Date.....

VS20 WAVE Complete Modular System

VS20WAVECES-HI, 20x20cm

Units include tank and lid **plus** the following accessories:

VS20CBS-HI	**PAGE Insert**	**Casting Base**	**Glass Plates**	**Combs**	**Cooling Coil**
	1x VS20WAVEDIRM	1x VS20WAVECAST	1x VS20PGS1 1x VS20NG	2x VS20-24-1	1x VS20WAVE-CC
	Blotting Insert	**Cassettes**	**Fibre Pads**	**Cooling Pack**	**Cables**
	As per VS20BI-HI			1x VS20CB	1x CSL-CAB
	WAVE Maxi Tube Gel Insert, *complete with glass tubes and blanking ports*			**IEF Conversion Kit**, *for 18cm IPG strips and Tube Gels*	
	1x VS20WAVEDCI			1x VS20WAVE-IEFKIT	

Packed & 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. 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 omniPAGE 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 omniPAGE Blotting 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.

Protein Blotting using the VS10BI-HI and VS20BI-HI

Hi Intensity Blotting Inserts

Setting up the blot sandwich: The most commonly used buffer solutions are given on page 11.

1. Each blot sandwich should be set up as follows:-

   a. Cassette clamp -ve (black) side placed in a tray or other suitable surface.

   b. Fibre pad, pre-soaked in transfer buffer. (An extra pad, also soaked in buffer, may be used to maximize compression.)

   c. At least two pieces of filter paper, pre-soaked in buffer. (The CSL-BP1010 and CS-BP2020 blot-absorbent filters papers are recommended respectively for the 10x10cm VS10BI-HI and 10x10cm VS20BI-HI inserts.)

   d. Gel.

   e. Transfer membrane. Usually, this requires pre-soaking, but consult the manufacturer's instructions for the type of membrane you are using. This should be smoothened out using a 25ml pipette or rolling pin to ensure that no air bubbles are trapped between the membrane and gel, thereby inhibiting transfer.

   f. Two pieces of filter paper, pre-soaked in buffer.

   g. Pre-soaked fibre pad.

   h. Cassette clamp +ve (red) side slotted into the groove in the bottom of the black cassette.

2. Close the hinge carefully so as to not disturb the sandwich.

3. Fill the tank with buffer solution up to the **maximum fill line** indicated on the side of each unit. See **BUFFER SOLUTIONS** section for recommended buffer solutions. Improved transfer can usually be obtained by using chilled buffer, or one the active or passive cooling options supplied (See **COOLING OPTIONS**).

Table 1. shows the volume of buffer required for each unit.

Buffer Volume	SB10-HI, CVS10CBS-HI	SB20-HI, VS20CBS-HI, VS20WAVECES-HI
One Cassette	1380ml	5600ml
Two Cassettes	1290ml	5240ml

COOLING OPTIONS

PASSIVE – COOL PACKS

Cooling is recommended for high intensity transfers because of the high electrical currents used. Cooling is also necessary to maintain the low temperatures important for protein stability during native transfers.

The cool packs supplied should be stored in the freezer and kept frozen for immediate use.

The VS10CB and VS20CB cool packs provide an ideal low-cost, cooling option for those laboratories not equipped with a recirculating chiller or without a laboratory water supply.

Each Cooling pack will take the place or 100ml of buffer for SB10 and 500ml of buffer for SB10W and SB20.

1. Fill the tank with the appropriate volume of transfer buffer, pre-chilled at 4°C.
2. Simply insert the cool pack into the tank after the blotting insert.

ACTIVE – COOLING COIL (SW20-HI, WAVECBS-HI, WAVECES-HI only)

To counteract the adverse effects of heat generation, the manufacturer recommends using an effective chiller unit, such as the CSL-CHILLER, preset to 4°C.

1. For active temperature regulation attach two short lengths of hose from the inlet and outlet ports of the chiller unit to the respective outlet and inlet connectors of the cooling coil sitting within the WAVE outer tank.

3. Ensure that the flow rate of the chiller unit is set at a maximum 1L/minute. Do not exceed this limit as excessive pressure might cause the hose to detach from the cooling coil.

4. If the chiller unit exceeds this limit, a T-connector may be used. The T-connector can divert some coolant volume back to the recirculating chiller reducing the pressure incident within the coil.

The unit is now ready to be used with active cooling.

Blot Run Conditions:

1. Insert the cassettes into the slots in the module with the black side of each adjacent to the negative electrode. It is a good idea to note the orientation and order the blot sandwiches were loaded in. This can be done by noting which samples were loaded adjacent to each electrode.
2. Use of a magnetic stirring bar and plate is recommended to mix the buffer to give consistency of transfer. A 4mm diameter stirring bar should be placed underneath the module, in the centre of the tank, which is located on a magnetic stirring plate (e.g. CSL-STIR). The cooling pack provided, pre-frozen, can be inserted at the side or front of the tank for extended blots. Additional cooling packs can be purchased as accessories to further aid cooling.
3. Insert the module, fit the lid and connect to a power supply.

   N.B. Because high intensity transfers require high current the CS-3AMP power supply, with a maximum 3000mA current output, is recommended.
4. Consult Table 2 for details on recommended power supply voltage settings and transfer times. Please note voltages and current will vary according to the number of cassettes, the type and temperature of buffer, and the thickness and percentage of gel. This will also affect quality of transfer so a time course for transfer should be performed for your particular samples - if you can spare them. Alternatively undertake the same time course using protein markers (e.g. CSL-PPL or CSL-BBL).
5. When the transfer time is completed, turn the power supply off.
6. Remove the cassettes from the main tank. Buffer can be re-used but this may adversely affect run quality if re-used excessively.
7. Lift the hinge of each cassette and gently pry apart the blot sandwich and remove the membrane from the gel.
8. The membrane is now ready to be probed.

Table 2. Recommended voltages and average resultant current.

Duration of Blot	**SB10-HI, CVS10CBS-HI**	**SB20-HI, VS20CBS-HI, WAVECES-HI**
One to Two Hours	25-100V 400-1000mA	100V 1000-1600mA
• Current should be limited to a maximum of 2000mA to prevent overheating; a power supply capable of reaching these settings (e.g. CS-3AMP) should be used. Please note that for some very high molecular weight proteins it may be necessary to increase the transfer time to 5 hours. **Excessive currents may cause tarnishing of the plate electrodes over time. This may be circumvented to a degree by using lower current settings, the cool packs supplied and freshly made transfer buffer, pre-chilled at 4°C.**		

References:-

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

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

3. **Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications,** Towbin, J., Staehelin, T., and Gordon, J. (1979). Proc. Natl., Acad. Sci. USA, 76, 4350-4354.

4. **Blotting Techniques Ch.1, 7.10, p. 85-97. In: Gel Electrophoresis of Proteins,**
A Practical Approach, B.D.Hames and D.Rickwood, eds., IRL Press. (1990),

BUFFER SOLUTIONS:-

Towbin Buffer

25mM Tris,
192mM glycine,
20% methanol pH8.3,

Towbin Buffer SDS

25mM Tris
192mM glycine
20% methanol pH8.3
0.05-0.1% (w/v) SDS

Bjerrum and Schafer-Nielsen Buffer

48mM Tris
39mM glycine
20% methanol pH9.2

Dunn Buffer

10mM $NaHCO_3$
3mM $NaCO_3$
20% methanol pH9.9

Do not adjust the pH when making these buffers as this will cause blot over-heating.

The pH will vary according to the freshness of the reagents used.

TROUBLESHOOTING

Poor protein transfer	Transfer apparatus assembled incorrectly and proteins moving in the wrong direction • Gel/membrane sandwich may be assembled in the wrong order, or cassette inserted in wrong orientation. Check polarity.
	Western detection system not working or not sensitive enough • Include proper positive or negative control antigen. Consult kit manual. • Use protein markers with coloured reference bands during PAGE (e.g. CSL-PPL or CSL-BBL). • Stain gel with Coomassie, or stain membrane with Ponceau S (CSL-PSS).
	Transfer time too short – increase transfer time
	Power setting too low • Check current at beginning of run. Current may be too low for a given voltage setting (see Table 2). Increase current if necessary but do NOT exceed 2000mA. • Buffer may be prepared improperly – prepare new buffer and increase voltage.
	Charge-to-mass ratio incorrect for native transfers. • Proteins close to isoelectric point (pI). Change buffer pH so that it is at least 2 pH unit higher or lower than pI of protein of interest.
	Defective or inappropriate power supply used. • Check fuse of power supply. Ensure max. current output of power supply is at least 2000mA (eg. CS-3AMP).
	Excessive methanol restricting transfer. • Reduce methanol concentration to maximize protein transfer from gel, but without reducing concentration to the extent that it prevents binding to nitrocellulose. Alternatively reduce methanol concentration and switch to PVDF (CSL-FPADSPVDF).
Protein precipitating in gel	• Use SDS in transfer buffer (SDS can increase transfer efficiency, but it can also reduce nitrocellulose binding affinity and affect protein-antibody reactivity). • Remove alcohol from transfer buffer.

Swirls or missing bands; diffuse transfers	Poor gel-membrane contact. Air bubbles or excess buffer remain between membrane and gel. • Carefully remove air bubbles between gel and membrane using a rolling pin • Use more, or thicker, filter paper in gel-membrane sandwich (e.g. CSL-BP1010, CSL-BP2020) • Replace the fibre pads, as they degrade and remain permanently compressed over time.
	Membrane not fully wet or has dried out • White spots on nitrocellulose membrane indicate dry areas to which proteins will not bind. Ensure membrane is completely immersed in transfer buffer. • If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet. • If using PVDF, immerse membrane in methanol before soaking in transfer buffer.
	Problem with gel electrophoresis. • Poor gel polymerization, inappropriate running conditions, buffer contamination, excessive sample application all contribute to poor quality gels and transfers.
Gel cassette pattern transferred to blot	Contaminated fibre pads • Replace fibre pads or clean thoroughly. Contaminated transfer buffer • Replace buffer solutions.
Poor binding to membrane - nitrocellulose	Excessive methanol restricting transfer. • Ensure methanol concentration does not exceed 20% (v/v).
	Proteins may be transferring through nitrocellulose. • Use PVDF or smaller pore size (0.2µm) nitrocellulose (e.g. CSL-RNC2) • Overlay an extra piece of nitrocellulose over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Proteins <15kDa have reduced binding to 0.45µm nitrocellulose or may be washed from membrane during assays. • Use PVDF or nylon membrane, which have higher binding capacities. • Use Tween-20 detergent in the wash and antibody incubation steps. Reduce or eliminate the more

	stringent washing steps.
	SDS in transfer buffer reducing binding efficiency • Reduce or eliminate SDS concentration
	Membrane incompletely wet • White spots indicate dry areas where protein will not bind. • If soaking does not occur immediately following immersion in transfer buffer, heat distilled water to just below boiling point and soak membrane until entirely wet.
Poor binding to membrane PVDF	Membrane is not completely wet • Because of hydrophobicity of PVDF, the membrane must be soaked entirely in methanol before equilibration in aqueous buffer
	Proteins might be transferring through the membrane • Decrease voltage if transferring under high intensity conditions • Overlay an extra piece of PVDF over membrane to determine if proteins are migrating through the membrane directly in contact with the gel.
	Membrane might have dried during handling • Fully wet membranes have a grey translucent appearance. White spots will form on the surface if the membrane has been allowed to dry. As proteins will not bind to dry spots, re-soak the membrane in methanol and re-equilibrate in transfer buffer
	SDS in transfer buffer reducing binding efficiency • Reduce or eliminate SDS concentration
Power is too high	Always check current at the start of the run, for the current might be too high for a given voltage setting. Improper buffer preparation can also result in high conductivity and excessive power generation. The current setting should not be allowed to exceed 2000mA.
Immune-specific detection	Overall high background • Reduce antibody / protein sample concentration Too low background • Increase antibody concentration / protein sample concentration Consult manual included with antibody detection kit
Total protein detection	Consult stain or detection kit manual.

Warranty

The Cleaver Scientific Ltd. (CSL) 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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