Instruction Manual

WAVE Standard and Tetrad Electroblotting, 2-D and Complete Electrophoresis Systems

Electroblotting	
VS20WAVECBS AND WAVETETRAD1CBS Includes VS20WAVESYS and WAVEBI	
SW20 & WAVEBI	
2-D Electrophoresis	
WAVEC2DS Includes VS20WAVESYS and VS20WAVEDCI	
VS20WAVEDC	
Complete (Electroblotting & 2-D Electrophoresis)	
VS20WAVECES AND WAVETETRAD1CES Includes VS20WAVECBS and VS20WAVEDCI	

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.

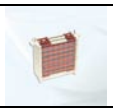
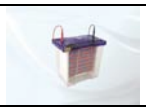
ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.

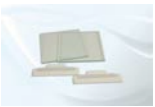
THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.
THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.
ACRYLAMIDE IS A POWERFUL NEUROTOXIN IN SOLUTION FORM.
POLYMERIZED GELS CAN CONTAIN SOME UNPOLYMERIZED SOLUTION AND PROTECTIVE GLOVES AND CLOTHING 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:

WAVECBS, SW20 models

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

	Glass Plates	Combs	Casting base	Cooling	Cables
WAVECBS Includes VS20WAVESYS And WAVEBI, plus cassettes & fibre pads	VS20NG - Notched, Pk/2 VS20PGS1 – Plain with bonded 1mm spacers, Pk/2 VS20-DP – Dummy Plate	2 of **VS20-24-1** 1mm thick, 24 sample	VS20DCAST VS20DCASTM - Mat	VS20ICB & VS20WAVE-CC cooling coil	CSL-CAB
	Internal Blotting Module	**Cassettes**	**Fibre pads**		
WAVEBI	Yes, plus…	4 of SW20C	2 of SB20F, Pk/6	(no tank, no lid)	
SW20	**WAVEBI** with cassettes and fibre pads				

	Glass Plates	Combs	Casting base	Cooling	Cables
VS20WAVEC2DS Includes VS20WAVESYS And VS20WAVEDCI & VS20WAVE-IEFKIT	VS20NG - Notched, Pk/2 VS20PGS1 – Plain with bonded 1mm spacers, Pk/2 VS20-DP – Dummy Plate	2 of VS20-24-1 1mm thick, 24 sample 2 of VS20-1-1.5 - 1.5mm thick, 1 sample	VS20DCAST VS20DCASTM - Mat	VS20ICB & VS20WAVE-CC cooling coil **Spacers** 2 of VS20S1.5	CSL-CAB
	Tube Gel Module	**Capillary Tubes**	**Blanking Plugs**		
VS20WAVEDCI	Yes, plus…	MCT20, pk/100	MCBP, pk/10		
VS20WAVEDC	**VS20WAVEDCI**	MCT20, pk/100	MCBP, pk/10		
VS20WAVE-IEFKIT IEF Conversion Kit for 18cm IPG strips and Tube gels	VS20PGS1/0.6 – Plain glass plates with 1mm bonded spacers (0.6x20cm WxH), Pk/2	2x 2D combs with one 3.5mm marker well and 18cm preparatory well			

	Glass Plates	Combs	Casting base	Cooling Pack	Cables
VS20WAVECES **Includes VS20WAVESYS** **And VS20WAVEDCI** **VS20WAVE-IEFKIT**	VS20NG - Notched, Pk/2 VS20PGS1 – Plain with bonded 1mm spacers, Pk/2 VS20-DP – Dummy Plate	2 of VS20-24-1 1mm thick, 24 sample 2 of VS20-1-1.5 - 1.5mm thick, 1 sample	VS20DCAST VS20DCASTM - Mat	VS20ICB **Spacers** 2 of VS20S1.5	CSL-CAB
	Tube Gel Module	**Capillary Tubes**	**Blanking Plugs**		
VS20WAVEDCI	Yes, plus…	MCT20, pk/100	MCBP, pk/10		
VS20WAVE-IEFKIT IEF Conversion Kit for 18cm IPG strips and Tube gels	VS20PGS1/0.6 – Plain glass plates with 1mm bonded spacers (0.6x20cm WxH), Pk/2	2x 2D combs with one 3.5mm marker well and 18cm preparatory well			
	Internal Blotting Module	**Cassettes**	**Fibre pads**		
WAVEBI blotting insert	Yes, plus…	4 of SW20C	2 of SB20F, Pk/6	Cooling coil & Pack	

Tetrad versions WAVETETRAD1CBS and WAVETETRAD1CES

	Glass Plates	Combs	Casting base	Cooling Pack	Cables
WAVETETRADCES **Includes VS20WAVESYS** **And VS20WAVEDCI** **VS20WAVE-IEFKIT**	1 of VS20NG – Notched, Pk/2 2 of VS20PGS1 – Plain with bonded 1mm spacers, Pk/2 2 of VS20NGS1 Notched with 1mm spacer, Pk/2 VS20-DP – Dummy Plate	4 of VS20-24-1 1mm thick, 24 sample 2 of VS20-1-1.5 - 1.5mm thick, 1 sample	1 of VS20DCAST (includes 1x VS20CASTM) **+ External CASTING STAND (no electrodes)** VS20WAVE-EC	VS20ICB **Spacers** 2 of VS20S1.5	CSL-CAB
	Tube Gel Module	**Capillary Tubes**	**Blanking Plugs**		
VS20WAVEDCI	Yes, plus…	MCT20, pk/100	MCBP, pk/10		
VS20WAVE-IEFKIT IEF Conversion Kit for 18cm IPG strips and Tube gels	VS20PGS1/0.6 – Plain glass plates with 1mm bonded spacers (0.6x20cm WxH), Pk/2	2x 2D combs with one 3.5mm marker well and 18cm preparatory well			
	Internal Blotting Module	**Cassettes**	**Fibre pads**		
WAVEBI blotting insert	Yes, plus…	4 of SW20C	2 of SB20F, Pk/6	Cooling coil & Pack	

	Glass Plates	Combs	Casting base	Cooling Pack	Cables
WAVETETRADCBS **Includes VS20WAVESYS**	1 of VS20NG – Notched, Pk/2 2 of VS20PGS1 – Plain with bonded 1mm spacers, Pk/2 1 of VS20NGS1 Notched with 1mm spacer, Pk/2 VS20-DP – Dummy Plate	4 of VS20-24-1 1mm thick, 24 sample	1 of VS20DCAST (includes 1x VS20CASTM) **+ External CASTING STAND (no electrodes)** VS20WAVE-EC	VS20ICB	CSL-CAB
	Internal Blotting Module	**Cassettes**	**Fibre pads**		
WAVEBI blotting insert	Yes, plus…	4 of SW20C	2 of SB20F, Pk/6	Cooling coil & Pack	

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 the WAVE unit

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 buildup 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
Ethanol	Methanol	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°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 WAVE 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.

VS20WAVESYS Set Up

Introduction

The new VS20 'WAVE' Maxi System is the latest product innovation for large-format vertical gel electrophoresis. Designed to perform a variety of separations, including first- and second-dimension SDS-PAGE, native, preparative, gradient and high-resolution nucleic acid electrophoresis, plus capillary tube gel IEF and electroblotting, the VS20 WAVE is one of the most versatile maxi vertical systems available.

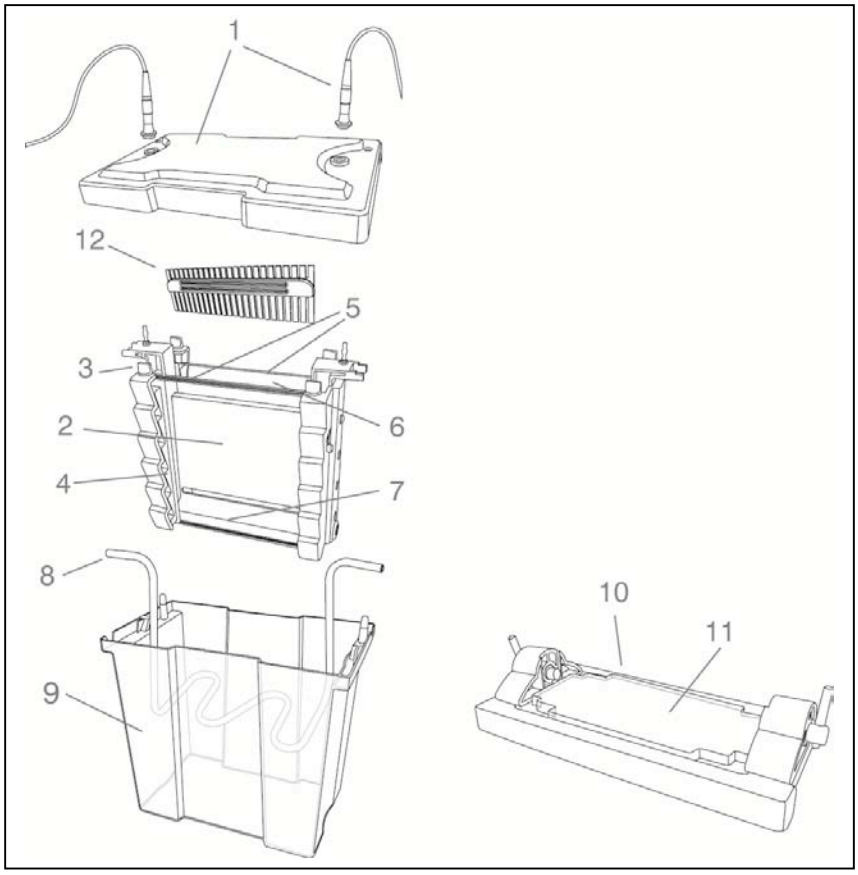
By introducing innovative, new vertical screw-clamp technology within the PAGE insert only four screws are now necessary to secure as many 20x20cm gels. This gives the VS20 WAVE Maxi the selective advantage of a much faster set up speed compared to competitor products whose traditional clamping configurations require as many as 24 screws to secure just two glass plates. In addition, the WAVE's vertical screw-clamp configuration distributes pressure evenly along the height of the gel rather than in the centre to eliminate plate bowing and gel compression, but still maintains a leak-proof seal during casting; while the ergonomic wave-like design of the PAGE insert aids both handling and set up.

Whatever your requirements are the WAVE can be made to meet them. Regardless of whether it is running 2 or 4 gels, electroblotting, and IEF using capillary tube gels or IPG strips, all of these techniques may be performed using the same omni-purpose unit while retaining the benefits of large format electrophoresis, such as extended separation distances, greater sample throughput and superior resolution.

Before using the WAVE we recommend assembling and disassembling the unit before using it for electrophoresis, and familiarising yourself with the component parts. Please see Figure 1 below.

Figure 1. WAVE Maxi Vertical Component Parts

WAVE Maxi Vertical Component Parts
1. Lid and power cables
2. PAGE insert
3. Vertical screw-pin
4. Clamping bars
5. Glass plates
6. Inner buffer chamber
7. Gasket
8. Detachable cooling coil
9. Outer tank
10. Cam-pin caster
11. Ultra-soft casting mat
12. Combs



Key Features

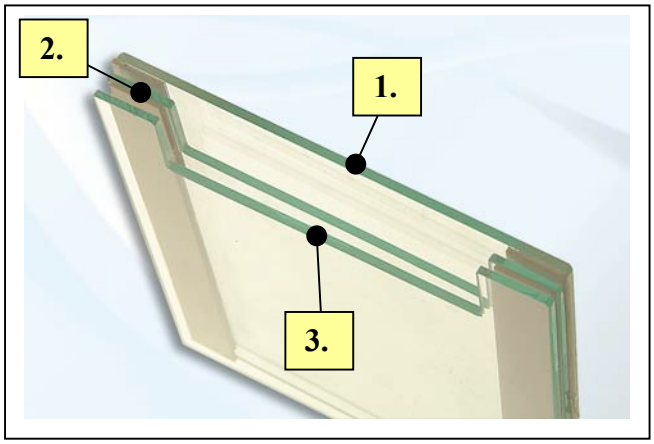
- **Vertical screw-clamp technology** - using only 4 screw-pins - dramatically reduces assembly time; also act in conjunction with colour-coded clamping bars (see below) to distribute pressure evenly along the height of the gel to prevent plate bowing or compression (Fig.1, number 3)
- **A built-in inner buffer chamber within the PAGE insert** - allows set up to be completed without inclusion of a top tank or upper buffer chamber (Fig.1, number 6)
- **Colour-coded clamping bars** – use green clamping bars to run up to two 2-mm-thick gels in the standard WAVE configuration, or by using the thinner, yellow clamping bars to run up to four 2-mm-thick gels in the WAVE TETRAD systems (Fig.1, number 4; also see section on **Converting the WAVE from a 2- to 4-gel Configuration**)
- **Glass plates** - compress gently against a flat, level gasket to prevent current leakage from the inner buffer chamber during electrophoresis (Fig.1, number 5); notched glass plate is placed innermost and compresses directly against the gasket to allow buffer to fill the upper wells within the gel, thereby facilitating electrical connectivity between the negative electrode and the top of the gel, whereas the plain glass plate with bonded spacers is outermost to prevent buffer leakage from the inner chamber
- **Detachable inner cooling coil** - connects to the laboratory water supply or a recirculating chiller to provide uniform, smile-free electrophoresis, while allowing runs to be performed at higher voltage (Fig.1, number 8)
- **Deep gel tank** - with adequate clearance beneath the glass plates to allow a magnetic stirrer to maintain buffer recirculation and uniform pH (Fig.1, number 9)
- **Cam-caster** - with very forgiving ultra-soft silicone mat compensates for glass plate misalignment to ensure leak-free casting (Fig.1, number 10 & 11)

Other Features

- Notched glass plates with bonded spacers supplied with WAVETETRAD systems allow gel capacity to be doubled from 2 to 4, while dummy plate allows single gels to be run (See Fig. 2)

Fig. 2. Glass plate set-up for 2-gel sandwich using yellow spacers

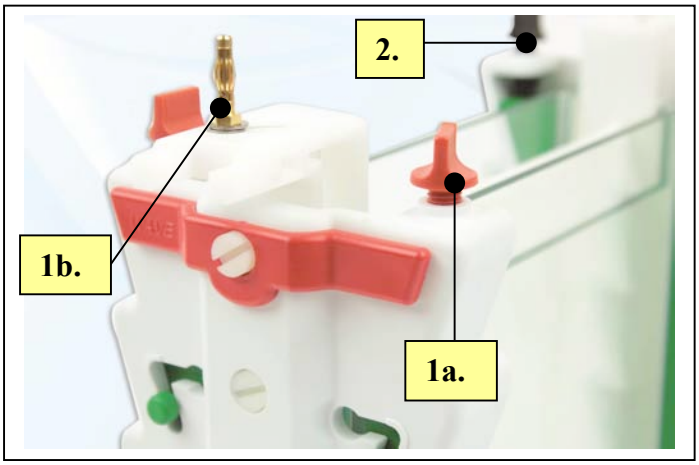
1. Plain glass plate with bonded spacer
2. Notched glass plate with bonded spacer
3. Notched glass plate



- Bonded spacers and combs colour coded for thickness (not shown)
- Asymmetric lid design (Fig. 1, number 1) and colour-coded screw pins in PAGE insert prevent polarity reversal (See Fig. 3)

Fig. 3. Colour-coded screw pins prevent polarity reversal

1. Red vertical screw pin (1a.) corresponding to positive electrode plug (1b.) colour-coded with **positive** power cable
2. Black vertical screw pin corresponding to negative electrode plug colour-coded with **negative** power cable



Leak-free Casting Using Vertical Screw-Pin Technology

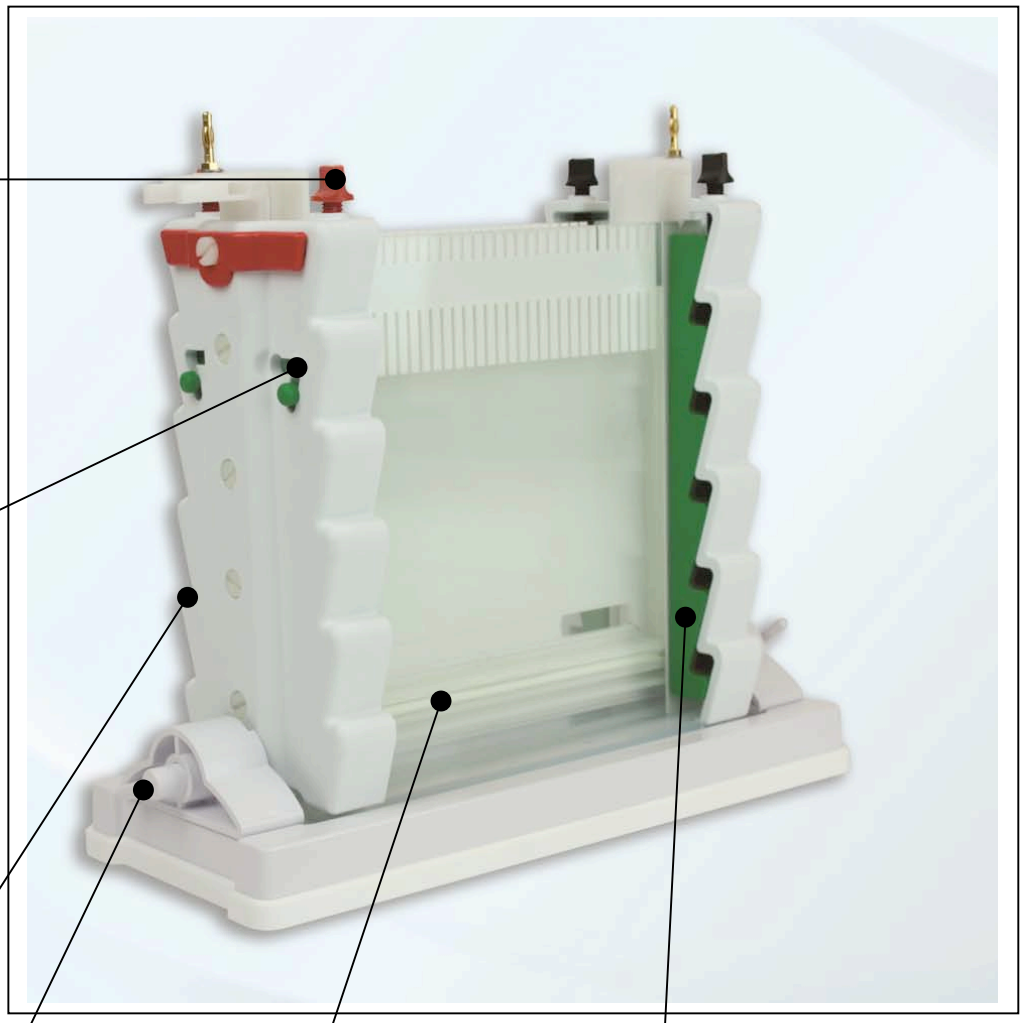
Fig. 4. The vertical screw-clamp technology of the VS20 WAVE PAGE insert facilitates fast, leak-proof gel casting.

Vertical screw-pins, colour-coded to prevent polarity reversal, push gel clamps out of the resting slots to secure glass plates firmly within the PAGE insert

Resting slots allow the gel clamps to sit conveniently out of the way, to aid hindrance-free loading of the cassettes into the PAGE insert

Ergonomic 'wave' design of PAGE insert provides convenient finger grips for easy handling

Cam pins lock PAGE insert onto the ultra-soft silicone mat within the casting base to provide leak-free seal



Flat, level gasket prevents current leakage from inner buffer chamber

Sliding gel clamps available in two thicknesses and colours to accommodate single- (green) and double-gel (yellow) cassettes

Vertical Gel Casting for 2 Gels Using the VS20WAVE and WAVETETRAD systems

General rules

1. Clean a set of glass plates for each gel first with distilled water and then with 70 % ethanol. One set of glass plates constitutes one notched glass plate and one plain glass plate with bonded spacers. When using a 2-gel glass plate sandwich in the WAVETETRAD to cast 2 gels either side of the PAGE insert, two notched glass plates are required - one with and the other without bonded spacers - as well as a set of plain glass plates with bonded spacers (Please also see section on **Converting the WAVE from a 2- to 4-gel Configuration**). The plain glass plate is positioned outermost, followed by a notched glass plate with bonded spacers and then a second notched glass plate (Please see **Fig. 2. Glass plate set-up for 2-gel sandwich using yellow spacers**).

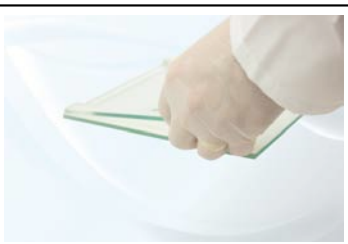
   N.B. - All glass plates, modules and casting base accessories must be completely dry during set – up. Wet components are more likely to misalign and cause leaks.

2. Assemble the glass plates so that the bottom of the glass plates and the spacers are perfectly aligned; the standard glass plates supplied with the WAVEDSYS, WAVEDSYS-CU and WAVETETRAD systems are supplied with bonded spacers and do not require manual alignment. However, if using free spacers, which are not included as standard, these need to be perfectly aligned. This is best performed using a small spacer or comb to push the spacers apart.

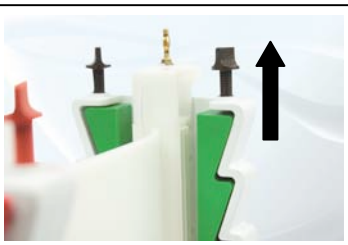
   NOTE: The glass plates with bonded spacers have an arrow in the top of the spacers which are slightly longer than the glass plate to indicate the top.

Please follow the illustrations below for detailed set up of WAVE system

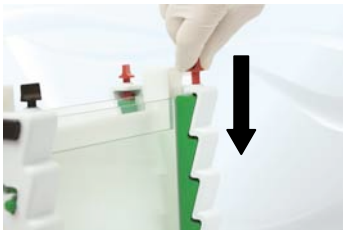
1. Assemble each gel cassette on a flat level surface, by placing the plain glass plate down with the spacers facing upwards followed by the notched glass plate.



2. Loosen the vertical screw-pins in the PAGE insert to release the locking mechanism, allowing the gel clamps to sit in the resting slots.

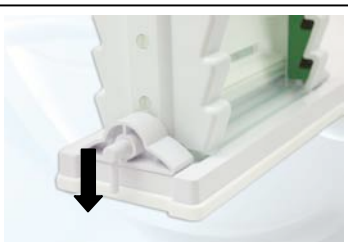


3. Insert a gel cassette into each side of the inner buffer chamber in the PAGE insert, and begin tightening the vertical screw-pins.



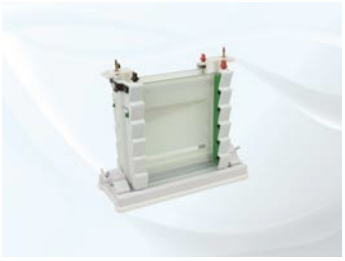
4. Continue to tighten the screw-pins until the gel clamps glide out of the resting slots and fix firmly against the glass plates pushing them upright.

5. Check the bottom of the glass plates to ensure that they are flush together, and place the PAGE insert on the casting base; make sure that the cams are facing downwards.



6. Insert cams and turn until tightened, drawing the PAGE insert onto the casting to form a leak-proof seal.

7. Pour in the gel solution, insert the combs and allow the wells to polymerise. Gel pouring is best performed using a 25ml or 50ml pipette.



8. Transfer the PAGE insert to the gel tank. Fill the inner and outer buffer chambers before loading samples.

9. Replace the lid, connect to the power supply and run.

Converting the WAVE from a 2- to 4-gel Configuration

The thicker green sliding gel clamps are recommended to secure up to 2 gels (i.e. 1 gel either side of the PAGE insert) for gels up to a maximum thickness of 2mm. For 4 gels (i.e. 2 gels either side of the PAGE insert: made using 1 plain glass plate and 1 notched glass plate, both with bonded spacers, and 1 notched plate without spacers), the thinner YELLOW sliding gel clamps **must** be used.

To convert the WAVE from a 2- to 4-gel configuration please adhere to the following instructions.

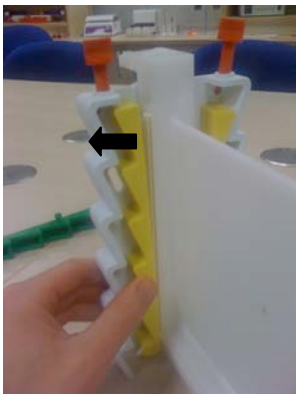
1. To replace the green sliding gel clamps, begin by unscrewing the colour-coded vertical screw pins. There should be no glass plates within the PAGE insert, in the side(s) being unscrewed. Once the screw pins are unscrewed sufficiently the green clamp should sit in the resting slot as shown.

2. Gently push the green sliding clamp horizontally towards the core of PAGE insert until it can move no further.

3. Push the green sliding gel clamp out of the PAGE insert by gently pressing the protruding pin as shown.

4. Once the green sliding gel clamp is removed the PAGE insert is ready to accept the thinner yellow sliding gel clamps for 2-gel sandwiches, either side of the PAGE insert, to convert the WAVE to a 4-gel configuration.

5. Insert the yellow sliding gel clamp into the hole closest to the core of the PAGE insert. Once inserted gently withdraw the sliding clamp outwards away from the core of the PAGE insert as shown.

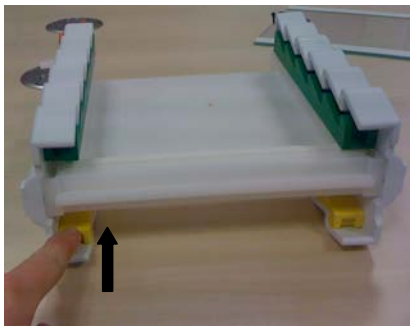


6. Withdraw the sliding gel clamps so that they sit suspended in the resting slots as shown. Repeat steps 1-6 to replace the remaining green sliding gel clamps. Once complete the PAGE insert is ready

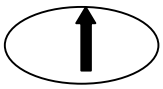
4-gel Assembly for 1.5- and 2-mm-thick Gels

Once converted into the 4-gel configuration using the yellow sliding gel clamps the WAVE is ready to accept and run a maximum of four gels ranging in thickness from 1mm to 2mm. 1-mm-thick 2-gel sandwiches may be inserted into the PAGE insert from the top as shown in Figures 1-9 in the ***Detailed set up of the WAVE insert*** section on pages 13-14. However, for1.5- and 2-mm-thick 2-gel sandwiches, it may be necessary to load the WAVE by positioning the PAGE insert on its side in order to overcome any resistance that may be posed by the gasket. The instructions below illustrate how best to undertake this.

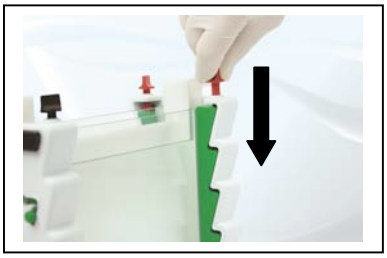
1. With the PAGE insert upright in its normal position, unscrew the screw pins sufficiently so that that the sliding gel clamps sit suspended within the resting slots. Turn the PAGE insert on its side and push the sliding clamps until they lie completely flat within the PAGE insert parallel with the bench surface.



2. Form a 2-gel sandwich on an even bench surface, as described in **General Rules** on page 12 in the Vertical Gel Casting Section. Push the 2-gel sandwich with the



3. Once the resistance is overcome push the uppermost notched glass plate into the PAGE insert. Restore the PAGE to its normal upright position and begin securing the glass plates for vertical electrophoresis, as described in Steps 3-9 of the **Detailed set up of the PAGE insert** on pages 13-14.



Gel Preparation:-

1. It is always advisable to work using stock solutions which allow added convenience and save time when it comes to gel pouring. Page 25 lists stock solutions for SDS PAGE gels which should be pre-made beforehand. For native gel formulae and running conditions, please consult a laboratory manual. The protocol below is given for use of the standard stock solutions advised. This should be adjusted if you are using different stock solutions or gel formulas.

2. Table 1 below shows the total volume of gel solution required. In subsequent tables, amounts of gel and solutions are given for two 1mm thick gels so adjustments are needed for when running single or more than two gels and for 0.75, 1.5 or 2mm thick spacers.

Table 1.

omniPAGE 'WAVE' – VS20WAVE, WAVEDSYS, WAVETETRAD	
	Total Gel volume for a 1mm thick gel.
For different thicknesses of gel, multiply the below amounts by the spacer thickness.	
Single – one gel, one dummy plate	35ml
Double – two gels	70ml
Using a Triple Plate 2-gel sandwich – four gels	140ml

Gel Selection:-

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

3. Prepare gel solutions as per tables below. These give the volumes of solutions from the standard stock solutions. These should be gently mixed avoiding generation of bubbles which will inhibit polymerization by removing free radicals.

Table 3: Preparation of the separating gel solution for two 20 x 20cm VS20WAVE gels using 1 mm spacers.

Solution	5 %	7.5%	10 %	12%	15 %	17.5%
Distilled Water	41ml	35.25ml	29.6ml	24.7ml	17.6ml	11.7ml
30 % Stock Acrylamide Solution	11.7ml	17.6ml	23.5ml	28.2ml	35.25ml	41.1ml
4 X Resolving Tris Solution	17.6ml	17.6ml	17.6ml	17.6ml	17.6ml	17.6ml
10 % Ammonium Persulphate	700µl	700µl	700µl	700µl	700µl	700µl

Gel Pouring:-

For gels with stacking layers:-

4. Insert the comb into the glass plates and mark a point on the glass plates 1cm below where the comb teeth finish. This indicates where to add the resolving gel to.

5. Add 70µl of TEMED to the gel solution and mix well, but avoid generating air bubbles.

6. Fill the glass plates again avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.

7. Overlay the gel extremely carefully with 1 ml of Isobutanol, Isopropanol or distilled water. When using distilled water extra care must be taken to ensure there is no mixing with the gel solution.

8. Let the resolving gel polymerize. Usually this takes around 15 minutes but this can vary due to the freshness of the reagents used. If polymerization is taken a lot longer than this, use fresher stock solutions or add more APS and TEMED.

9. Prepare the stacking gel using Table 4 below as a guide. Again stock solutions are given on pages 17 and 18. **Table 4.**

Solution	VS20WAVE
Distilled Water	16.8ml
30 % Stock Acrylamide Solution	2.6ml
4 X Stacking Gel Tris Solution	6.4ml
10 % Ammonium Persulphate	178 µl

10. Carefully mix the stacking gel solution, avoiding generating air bubbles.

11. Pour off the overlay liquid and rinse the gel with distilled water.

12. For VS20WAVE gels add 26.8µl of TEMED. Mix well. Use a Pasteur pipette to fill the glass plates up to the top with stacking gel solution.

13. Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.

14. Allow the stacking gel polymerize for 30 minutes.

For gels without stacking layers:-

4. Add 70µl of TEMED for VS20WAVE gels and mix well but avoid generating air bubbles.
5. Fill the glass plates again avoiding generating any air bubbles. Filling must be performed quickly before the TEMED causes the gel to become too viscous.
6. Carefully insert the comb making sure that no air bubbles get trapped under the ends of the comb teeth as these will inhibit sample progression.
7. Let the gel polymerize. Usually this takes around 15 minutes but this can vary due to the freshness of the reagents used. If polymerization is taken a lot longer than this, use fresher stock solutions or add more APS and TEMED.

Sample Preparation & Loading

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 28).
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 manufacturer's 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.

Loading the samples:

1. Transfer the Inner gel module containing cast gels into the main tank in the correct orientation as indicated - +ve on the module aligned with +ve on the tank, -ve on the module aligned with –ve on the tank.
2. Fill the outer tank with 1 x reservoir buffer. See page 22 for recommended running buffer solution. Table 6 shows the volume of buffer required.
3. Load the samples into the wells using a pipette tip taking care not to damage the wells or induce any air bubbles.
4. Fill any unused wells with 1 X sample buffer.
5. It is a good idea to note the orientation and order the samples were loaded in. This can be done by noting which samples were loaded adjacent to each electrode.

Buffer Volume:

Table 5.

Buffer Volume	VS20WAVE
Minimum – Inner tank is filled to above the wells. Outer Tank is filled to just flood the bottom of the glass plates. Cooling potential is at a minimum which may affect resolution.	Total Inner Buffer Chamber:: 640ml Outer Tank: 1L
Maximum – Inner tank is filled to above the wells. Outer Tank is filled to the maximum fill line. Cooling is high offering good resolution of samples. This may be further enhanced by using the cooling coil	Total Inner Buffer Chamber:: 640ml Outer Tank for 2 gels: 5.4L Outer Tank for 4 gels: 4.8L

Gel Running:

1. Fit the lid and connect to a power supply.

2. Consult Table 6, page 24 for details on recommended power supply voltage settings.

3. Turn the power supply off when the loading dye reaches the bottom of the gel, sooner if your proteins are below 4Kd in size.

4. Remove the gel running module, first emptying the inner buffer into the main tank. Buffer can be re-used but this may affect run quality if continued.

5. Unscrew the glass plates and gently pry apart the glass 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.

6. 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.

Table 6.

Recommended Voltages and Resultant Current for 1mm thick, 12% gels.	VS20WAVE
2-4 gels	35mA (constant); up to 350V maximum Runtime to 5h – no cooling; 4h with cooling

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

Add Distilled Water to a final volume of 200ml

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

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 Stacking Tris

0.03 g Bromophenol blue

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

Vertical Gel Electrophoresis 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.

Combs:–

MC Denotes Multi Channel Pipette compatible.

VS20WAVE, WAVESYS-CU, WAVETETRAD

Code	Description	Sample Volume µl Per Well
VS20-1-0.75	Comb 1 Prep, 1 Marker, 0.75mm thick	1100
VS20-5-0.75	Comb 5 sample, 0.75mm thick	160
VS20-10-0.75	Comb 10 sample, 0.75mm thick	80
VS20-18-0.75MC	Comb 18 sample MC, 0.75mm thick	40
VS20-24-0.75	Comb 24 sample, 0.75mm thick	30
VS20-30-0.75	Comb 30 sample, 0.75mm thick	25
VS20-36-0.75MC	Comb 36 sample MC, 0.75mm thick	20
VS20-48-0.75	Comb 48 sample, 0.75mm thick	15
VS20-1-1	Comb 1 Prep, 1 Marker, 1mm thick	1500
VS20-5-1	Comb 5 sample, 1mm thick	200
VS20-10-1	Comb 10 sample, 1mm thick	100
VS20-18-1MC	Comb 18 sample, 1mm thick	50
VS20-24-1	Comb 24 sample, 1mm thick	40
VS20-30-1	Comb 30 sample, 1mm thick	35
VS20-36-1MC	Comb 36 sample MC, 1mm thick	25
VS20-48-1	Comb 48 sample, 1mm thick	20
VS20-1-1.5	Comb 1 Prep, 1 Marker, 1.5mm thick	2200
VS20-5-1.5	Comb 5 sample, 1.5mm thick	320
VS20-10-1.5	Comb 10 sample, 1.5mm thick	160
VS20-18-1.5MC	Comb 18 sample, 1.5mm thick	80
VS20-24-1.5	Comb 24 sample, 1.5mm thick	60
VS20-30-1.5	Comb 30 sample, 1.5mm thick	50
VS20-36-1.5MC	Comb 36 sample MC, 1.5mm thick	40
VS20-48-1.5	Comb 48 sample, 1.5mm thick	30
VS20-1-2	Comb 1 Prep, 1 Marker, 2mm thick	3000
VS20-5-2	Comb 5 sample, 2mm thick	400
VS20-10-2	Comb 10 sample, 2mm thick	200
VS20-18-2MC	Comb 18 sample, 2mm thick	100
VS20-24-2	Comb 24 sample, 2mm thick	80
VS20-30-2	Comb 30 sample, 2mm thick	70
VS20-36-2MC	Comb 36 sample MC, 2mm thick	50
VS20-48-2	Comb 48 sample, 2mm thick	40

Connecting to a Chiller Unit

1. Vertical PAGE may sometimes result in high currents. High current may cause the build-up of heat which can affect sample migration and gel resolution as a result. 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.
2. 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 damage to the cooling platform might occur.
4. If using a chiller unit which 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.

Protein Blotting using the WAVE

(N.B. - If using SW20-HI, VS20WAVECBS-HI or VS20WAVECES-HI, please consult or request the high intensity blotting manual, obtainable from www.cleaverscientific.com or info@cleaverscientific.com)

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.

   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	SW20, WAVECBS,
One Cassette	6400ml
Two Cassettes	6040ml
Three Cassettes	5680ml
Four Cassettes	5320ml

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 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 of 500ml of buffer.

1. Simply insert the cool pack into the tank after the blotting insert.

ACTIVE – COOLING COIL

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		SW20, WAVECBS
One to Two Hours Three or more hours		100V 400mA 50V 200mA
• 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.		

Blotting 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	Poor gel-membrane contact. Air bubbles or excess

bands; diffuse transfers	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. • Use extra fibre pads and thicker blotting paper to enhance compression
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	Consult stain or detection kit manual.

detection	

1St Dimension Electrophoresis using the WAVEDCI Tube Gel Module

Capillary Tube Gel Pouring:-

There are two methods which can be used for tube gel casting. Method 1 details casting by injection; method 2 details casting by capillary action.

Method 1:- Filling By Injection

1. Place the appropriate number of capillary tubes into the Tube Gel Running module, inserting these carefully from the top.
2. Seal the bottom ends of the tubes using NescoFilm.
3. Prepare the following solution. This will be enough to pour twenty 80mm Capillary Tubes or ten 170mm Capillary Tubes. For Native IEF Gels, do not use Urea and NP-40 and use 18ml of distilled water instead of 16ml;

16ml Distilled Water (18ml for Native Gels)
2.4ml Glycerol
0.9ml 4-8 Resolyte or other commercially available 40% ampholyte solution
3.8ml Acrylamide/Bis solution
15µl TEMED
16.2g Urea (omit for Native Gels)
0.6ml NP40 (omit for Native Gels)

This solution should be de-gassed prior to pouring.
When ready to pour, add 120µl of 10% w/v ammonium persulphate solution.

4. Using a Hamilton or similar syringe, insert the needle into the tube and carefully inject the solution so that the tubes fill from the bottom. Keep filling to 1cm of the length of the tubes. The tubes can be gently tapped to get rid of air bubbles.
5. Fill the remaining 1cm gap with water saturated isobutanol.
6. Leave to fully polymerise, which will normally take 1 – 2 hours.
7. After polymerisation, remove the water-saturated isobutanol. Tube gels can be used immediately or stored wrapped in a damp paper towel and Nescofilm at 4°C. The Nescofilm at the bottom of the tubes must be removed prior to electrophoresis.

Method 2:- Filling By Capillary Action

1. Place the appropriate amount of capillary tubes in a suitable outer receptacle such as a 15ml falcon tube.

2. The amount of acrylamide required depends on the size of the outer receptacle used. The larger the outer receptacle used, the more acrylamide wastage so the following advised volumes may need to be increased.

Prepare the following solution. This will be enough to pour twenty 80mm Capillary Tubes or ten 170mm Capillary Tubes. For Native IEF Gels, do not use Urea and NP-40 and use 18ml of distilled water instead of 16ml;

32ml Distilled Water (18ml for Native Gels)

4.8ml Glycerol

1.8ml 4-8 Resolyte or other commercially available 40% ampholyte solution

7.6ml Acrylamide/Bis solution

30µl TEMED

32.4g Urea (omit for Native Gels)

1.2ml NP40 (omit for Native Gels)

This solution should be de-gassed prior to pouring.

When ready to pour, add 240µl of 10% w/v ammonium persulphate solution.

3. Fill the falcon tube with 70% of the acrylamide solution. The capillary tubes will fill by capillary action.

4. Allow the tubes to equilibrate for a few moments.

5. Check the height of the acrylamide in the tubes. If the tubes are full so that there is less than a 1cm non-filled space at the top, remove some of the acrylamide solution from the beaker until the height is 1 cm from the top. If there is a greater than 1cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1cm space at the top.

6. When the solution has reached to within 1cm of the top of the tube, stop adding the acrylamide solution.

7. Fill the remaining 1cm gap with water saturated isobutanol.

8. Leave to fully polymerise, which will normally take 1 – 2 hours.

9. After polymerisation, remove the water-saturated isobutanol. Tube gels can be used immediately or stored wrapped in a damp paper towel and Clingfilm at 4°C.

10. The tubes may contain a residual of acrylamide on the outside and may need cleaning with distilled water before insertion into the tube gel insert.

1st Dimension (IEF) Phase Tube Gel Running

Buffer and run conditions will vary according to the type of ampholyte used. The following conditions are given as guidelines only and apply when 4-8 Resolyte is the ampholyte used. Other Ampholytes will require different buffer solutions. Please consult manufacturer's instructions.

1. Prepare ~ 500ml of 10mM H3PO4 Anode Buffer (1 litre for VS10W, 2 litres for VS20) and use this to fill the bottom chamber of the unit so that the bottoms of the capillary tubes are submerged. If less than 10 capillary tubes are to be run, block up the unused tube slots in the internal running module with the blanking plugs provided. For high resolution separations, we recommend filling the lower chamber completely with buffer and using a pre-frozen cooling pack(s).

2. Place the Internal running Module into the unit and fill the upper buffer reservoir with ~100 mls of 20mM NaOH Cathode Buffer (200 ml for VS10W, 400mls for VS20) so that the tops of the capillary tubes are submerged.

3. For the Prefocus, load the gels with 10µl of 1% ampholyte solution and run for 15 minutes at 200V, then for 30 minutes at 300 V and then finally 30 minutes at 400V. The Prefocus stage is recommended as it helps set up the pH gradient.

4. Load the tubes with the samples. These should be dissolved in 1% ampholyte with 20% glycerol.

5. Replace the safety lid firmly making sure that the electrical connectors form a good contact.

6. Connect the electrophoresis apparatus to the power pack and connect the power pack to the mains supply. Turn all settings to zero before turning on the mains supply.

7. Run at 400V for 3 hours and then 800V for 30 minutes. These conditions are for 8cm tubes. 17cm tubes need to be run at 400V for 18 hours and then 800V for 1 hour.

8. At the end of the run, turn the power supply settings to zero, turn off the mains supply and disconnect the power leads.
9. Remove the Internal Module and remove the tubes from their slots. The gels can be extracted from the capillary tubes by: **a)** inserting a piece of wire with a small plug of cotton wool on the end and using this as a piston to push the gel out, **b)** inserting a Gilson tip into the end of the gel and gently
squeezing the gel out with air or water. Whichever of these two methods is used, the gels should be handled with care as they are fragile.

2-D, Size Determination Phase

1. To prepare the tube gel(s) for the 2-D, size-determining phase, equilibrate them by soaking for 30 minutes in the running buffer to be used for the 2-D phase.
2. Remove the gel(s) from the running buffer pre-soak, and place each lengthways onto the top of a pre-poured slab gel. The slab gel should be cast using a blank or 2-D comb. For details on the casting of slab gels see the section, ***Leak-free Casting Using Vertical Screw-Pin Technology***. Use the VS20PGS1/0.6 plain glass plates with 0.6x20cm (WxH) bonded spacers if using 18cm IPG Strips for 1st dimension IEF.
3. Hold the tube gel in place by pouring over it a low % agarose gel containing the tracker dye.
4. Electrophorese as usual for Slab Gels until the tracker dye has advanced the required distance down the gel.
5. The samples can be visualized using any of the standard staining methods or can be blotted.

NOTES

Warranty

The WAVE electrophoresis unit has 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 in which inappropriate 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 4 Triton Park
Swift Valley
Brownsover Road
Rugby
CV21 1SG
Tel: +44 (0)1788 565300
Fax: +44 (0)1788 552822
Email: info@cleaverscientific.com

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