DGGE System

Denaturing Gradient Gel Electrophoresis System

Instruction Manual

Catalogue Numbers

VS20WAVE-DGGE

VS20WAVE-DGGE$

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.

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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 List

Part No.	Description	Quantity
VS20WAVEDIRM	Inner Running Module	1
VS20WAVE-TANK	Main Tank	1
VS20WAVE-DGGETC	Temperature Controller	1
GM100	Gradient Mixer	1
VS20WAVELID	Tank Lid	1
VS20NG	Notched Glass Plates (2/pk)	1
VS20PGS1	Plain Glass plates, 1mm spacers (2/pk)	1
VS20-24-1PC	24 well comb, 1mm thick, polycarbonate	2
VS20WAVECAST	Casting Base	1
VS20DCASTM	Silicon Casting Mat	1
CSL-CAB	Power Cables	1

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

WAVE electrophoresis insert and tank	
Max. Number of Gels	2 per run
Plate Dimensions (W x H)	20 x 20cm
Active Gel Dimensions (W x H)	16 x 17.5cm
Spacer Thicknesses	0.75, 1, 1.5 or 2mm
Max. Sample Capacity	96 samples; 48 per gel
Standard Combs	2 x 1mm 24-sample, polycarbonate
Available Comb capacities	1, 5, 10, 18MC, 24, 30, 36MC, 48
Max. Buffer Volume	8.5L
Unit Dimensions	40.5x17x44cm (WxDxH)
Weight	8Kg
Recommended Power Supply	POWERPRO500
Temperature Control Unit	
Temperature Control	PID
Operating Temperature Range	Ambient-100°C
Working Temperature Range (DGGE)	45-70°C
Buffer Recirculation Mechanism	Stirring
Temperature Uniformity/Stability at 37°C	±0.05/0.02°C
Setting/Display Resolution	0.1°C
Stored Temperature Values	4
Safety	Fluid-level float switch; isolated; IEC 1010 / CE
Heater Power at 230V/110VAC	1.4/1.3kW
Electrical Power at 230V/100VAC	1.5/1.4kW (50-60Hz)
Gradient Mixer	
Total Volume	100ml
Volume of Reservoir and Mixing Chambers	50ml
Internal Diameter of Outlet Port	2mm

Operating Instructions

Further information (including videos) regarding setting up and running the DGGE units can be found at www.cleaverscientific.com

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°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 DGGE Gel Tank

Note: Before setting up the Gel Tank please ensure that it has been properly cleaned and dried.

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 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 and the unit is now ready to be used.

The unit is now ready to be used.

Casting Unit Preparation

Cleaning the Glass Plates

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 triple glass plate sandwich, two notched glass plates are required, one set of free spacers and a set of plain glass plates with bonded spacers. The plain glass plate is positioned outermost, then a notched glass plate, free spacers and second notched glass plate. Alternatively, accessory notch glass plates with bonded spacers are available.

Note: All glass plates, gel casting modules, casting base and accessories must be completely dry before the set – up. Wet components are more likely to miss-align and cause leaks.

Gel Cassette Assembly

Assemble the glass plates so that the bottom of the glass plates and the spacers are perfectly aligned.

A triple plate sandwich can be used by combining the standard Notched plate and spacer plate, with an additional notched plate with spacers. For triple plate sandwiches, the free spacers Need to be perfectly aligned which is best performed using a small spacer or comb to push the spacers apart. Notched glass plates with bonded spacers do not need manual alignment.

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.

Casting Stand Assembly

1. Ensure that the clamps are adequately open for the thickness of spacer used. The clamps can be opened by loosening the screws and sliding the clamps.

2. Position the Slab Gel Insert on a flat surface. Insert the glass plates into the Slab Gel Insert between the clamps and the white gasket. The Slab Gel Insert contains clamps which impart even pressure onto the glass plates and allow even screw pressure transfer onto the sealing edge of the glass plate, ensuring complete sealing.

3. Fully tighten the screws, making sure not to wobble the unit.

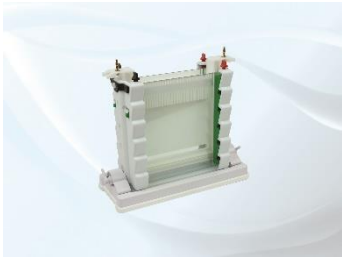
4. When only one gel is being run, the dummy plate must be used in the second position and fully tightened.

5. Position the Slab Gel Insert in the casting base such that the Cam pins have handles pointing downwards and are located in the insert holes. The top of the module may need to be pushed down very slightly to locate the cam pins.

NOTE: At this stage, check that the bottom edges of the spacers and glass plates are perfectly aligned.

6. With the cam pin handles facing directly downwards, turn the cam pins fully through 135° or until the insert has tightened onto the silicone mat.

NOTE: It is best to turn the cams in opposite directions to each other. Do not overturn as this will cause the glass plates to push upwards and the assembly will be more likely to leak. The unit is now ready for gel preparation and pouring.



7. Pour in the gel solution (see subsequent section for preparation of gradient gels), insert the combs and allow the wells to polymerise. If using a stacking gel, don't insert combs, allow gel to polymerise, pour stacking gel and then insert combs.

Casting 4 gels

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.

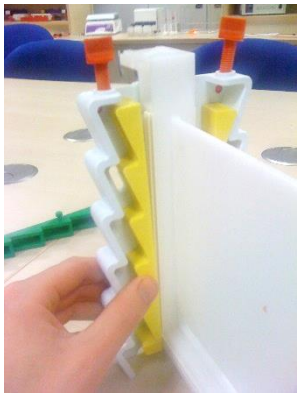
To convert the unit from a 2- to 4-gel configuration please adhere to the following instructions. One completed, proceed with casting as above.

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 for 4-gel assembly.

Gel Preparation for Parallel DGGE

The concentration of denaturant to use within a parallel DGGE gel depends on the sample being analysed within the VS20WAVE-DGGE system. A relatively narrow denaturant gradient range is applied, usually 25 to 30%, although the extremities of a parallel denaturing gradient gel may be as low as 10% and as high as 70%. The concentration of acrylamide may also vary, depending on the size of the fragment analysed. Both 0% and 70% denaturant should be made as stock solutions, based on a 100% denaturant solution containing a mixture of 7 M urea and 40% deionized formamide.

Stock Solutions

40% Acrylamide/Bis (37.5:1), 100ml

WARNING: Acrylamide is a neurotoxin, and especially dangerous when used in powdered form! Always wear a mask to prevent inhalation, or better still use commercial acrylamide solutions.

50x TAE Buffer, 1L. This may be purchased using code TAE50X1L or made up as follows:

Reagent	Amount	Final Concentration
Tris base	242g	2M
Acetic Acid, glacial	57.1ml	1M
0.5M EDTA, pH 8.0	100ml	50mM
Distilled water	To 1000mL final volume.	

Mix and, preferably, autoclave for 20-30 minutes. Store at Room Temperature.

Please consult the table below for the recommended % acrylamide/ bis required for a particular DNA size range.

Gel Percentage	Base pair Separation (bp)
6%	300-1000bp
8%	200-400bp
10%	100-300bp

0% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Distilled water	83ml	78ml	73ml	68ml
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C.

To make 100ml 70% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Formamide (deionized)	28ml	28ml	28ml	28ml
Urea	29.4g	29.4g	29.4g	29.4g
Distilled water	To 100ml			
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C. It may be necessary re-dissolve the denaturant after cold storage. If so, place the bottle within a 37°C water bath, preferably stirring the contents for faster dissolution (e.g. SWB-10L).

For 100ml denaturing solutions of maximum concentration less than 70%, the formamide and urea content may be adjusted as follows:

Denaturing Solution	10%	20%	30%	40%	50%	60%
Formamide	4ml	8ml	12ml	16ml	20ml	24ml
Urea	4.2g	8.4g	12.6g	16.8g	21g	25.2g

1ml, Ammonium Persulphate (APS) 10%.

Dissolve 0.1g APS in 1ml distilled water. Store at -20°C for 1 week.

Gradient Dye Solution

Reagent	Amount	Final Concentration
Bromophenol blue	0.05g	0.5%
Xylene cyanol	0.05g	0.5%
1x TAE buffer	10ml	1x

Store at room temperature.

2x Gel Loading Dye

Reagent	Amount	Final Concentration
2% Bromophenol blue	0.25 ml	0.05%
2% Xylene cyanol	0.25 ml	0.05%
100% Glycerol	7.0 ml	70%
dH2O	2.5 ml	
Total volume	10.0 ml	

Store at room temperature.

Gel Volumes

The table below provides the required gradient volumes to make one 16x17.5cm (width x height) parallel denaturant gradient gel in the VS20WAVE-DGGE inner running module, using the GM100 gradient mixer. The volume per chamber represents the volumes required for the high percentage mixing (A) and low percentage reservoir (B) chambers in the gradient mixer. To make gradient gels, using a gradient mixer, it is advisable to make one gel at a time, before washing out the apparatus and setting it up again to prepare a second gradient gel.

The volume per chamber requires a surplus volume of denaturant mixture, because the excess volume in each chamber is necessary to ensure that the correct volume of gel solution enters and fills the gel sandwich. An excess gel volume also accounts for the volume required to fill the silicon tube.

For CDGE use the entire gel volume.

Spacer thickness	Volume per 16 x 17.5cm gel	Volume per chamber
0.75mm	28ml	14ml
1mm	34ml	17ml
1.5mm	48ml	24ml

Gel preparation for CDGE

For CDGE gels, a 100% denaturing solution replaces the 70% solution as follows:

To make 100ml 100% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Formamide (deionized)	40ml	40ml	40ml	40ml
Urea	42g	42g	42g	42g
Distilled water	To 100ml			
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C. It may be necessary re-dissolve the denaturant after cold storage. If so, place the bottle within a 37°C water bath, preferably stirring the contents for faster dissolution (e.g. SWB-10L).

To cast constant denaturing gradient gels, use the formula below to determine the volume of 0% and 100% denaturing solutions needed to achieve the desired denaturant concentration.

1. (% desired denaturant) (total gel volume needed) = ml of 100% denaturant solution
2. (total gel volume needed) - (ml of 100% denaturant) = ml of 0% denaturant solution

Example: To cast a 56% constant denaturing gel, use 32 ml total volume for a 16 x 17.5 cm gel with a 1.0 mm bonded spacer.

1. (0.56)(32 ml) = 17.9 ml of 100% denaturing solution needed
2. (32 ml) - (17.9 ml) = 14.1 ml of 0% denaturing solution needed

Gel volumes are outlined above in the Parallel DGGE section.

Sample Preparation

Before performing DGGE, the PCR reaction should be optimised to ensure that the samples are free of any unwanted PCR products which might interfere with gel analysis. PCR products can be evaluated for purity by agarose gel electrophoresis before being loaded onto a denaturing

acrylamide gel. Cleaver Scientific recommends the MSCHOICE and MSMIDI96 units for checking PCR products (please visit www.cleaverscientific.com).

For a parallel denaturing gel, load 180-300ng of amplified DNA per well (usually 5-10% of a 100µl PCR volume from a 100ng DNA template). A wild-type control should be run on every gel.

Add an equal volume of 2x loading dye to the sample.

Heteroduplexes, if required, can be generated in two ways. The first method is to amplify both the wild-type and mutant DNA samples in the same tube during PCR, whereas in the second method both the wild-type and mutant samples may be amplified in separate tubes and heteroduplexes then formed by mixing an equal amount of wild-type and mutant within one tube, which is heated at 95°C for 5 minutes and stabilised at 65°C for a further hour, before allowing to cool slowly to room temperature.

Pre-heating Running buffer

Temperature controller operation

The VS20WAVE-DGGETC temperature controller, shown below, maintains the desired temperature of the VS20WAVE-DGGE during electrophoresis. VS20WAVE-DGGETC temperature control unit combines buffer recirculation with a heat sensor and 1.4kW heating element to facilitate precise temperature control to within ±0.2°C. This allows the gel temperature to be set to the melting temperature (Tm) of the amplified polymorphism or mutation of interest. However, in the interests of safety and successful operation a few moments should be spent becoming acquainted with the features of the VS20WAVE-DGGETC before using it.

Features

1. Alarm Light
2. Display
3. F (function) Button
4. Pump Outlet Plate *(TC120 only)*
5. Heater On Light
6. Over-Temperature Dial *(TC120 only)*
7. S (select) Button
8. Dial
9. Float: low liquid level protection

CAUTION! SAFETY CUT OUT AND ALARM LIGHT

The VS20WAVE-DGGETC is fitted with a float switch (9) that monitors the level of liquid during operation. If the liquid level within the VS20WAVE-DGGE tank were to fall below the level of the float switch during operation, the safety cut out would be activated causing the alarm light to appear. If this happens, disconnect the VS20WAVE-DGGE tank from the power supply, before removing the lid and adding sufficient buffer to re-immerse the float switch and resume electrophoresis.

NEVER OPERATE the temperature controller unless the buffer level at least covers the float switch.

Over-Temperature Dial (6)

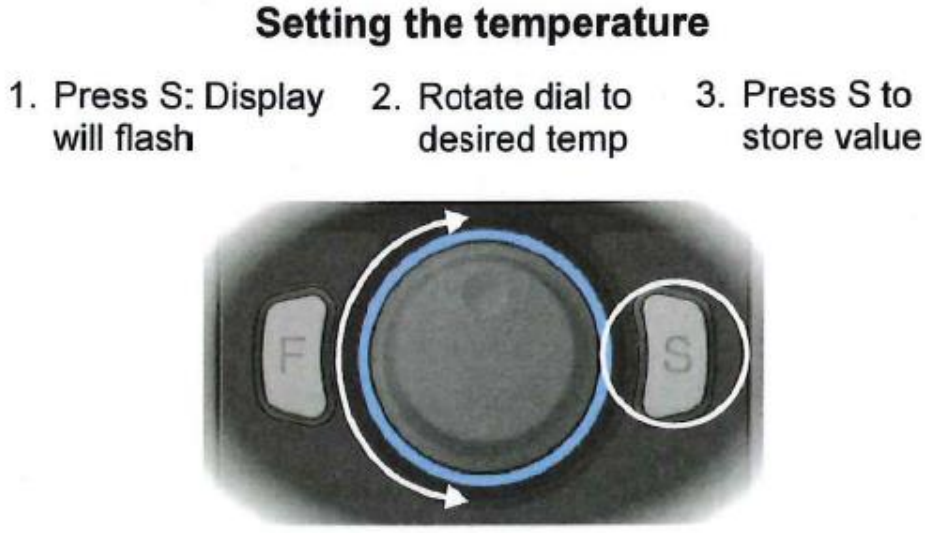
The over-temperature dial is factory pre-set for safety. Since a maximum temperature of 65°C is generally accepted for denaturing gel electrophoresis within the scientific literature, it should not be necessary to set the over-temperature dial in excess of 70°C. The over-temperature dial may be readjusted by gently turning the dial to the desired setting using a flat-bladed screw driver.

N.B. Heat damage to the unit from excessive temperatures will invalidate the warranty.

Adjusting the buffer temperature

Setting the buffer temperature is simple:

1. Press S (7) – the display will flash
2. Rotate the Dial (8) to the desired buffer temperature – e.g. 65°C
3. Press S to store the temperature value – the temperature is now set at 65°C, and the heater light will come on to show that heating is underway.
4. Allow the buffer to reach temperature (i.e. 65°C in this example).



Preheating the buffer

1. Fill the VS20WAVE-DGGE electrophoresis tank with 7 L of 1x TAE running buffer.

N.B. Linear and double-stranded DNA migrates faster in TAE buffer, although buffering capacity becomes depleted particularly during extended runs. Consequently, it is advisable to avoid reusing TAE buffer, as the migration rate and band resolution of DNA molecules may be adversely affected in subsequent DGGE experiments.

2. With the VS20WAVE-DGGETC temperature control unit in situ within the electrophoresis tank, connect the power cord to the temperature control unit and turn the power, pump, and heater on. The lid should be replaced on the VS20WAVE tank during preheating, to prevent evaporation and accidental scalding.
3. Set the temperature controller to the desired temperature as described in the section Temperature Controller. Set the desired temperature.
4. Preheat the buffer to the set temperature, which may take 1 to 1.5 hours. Heating the buffer in a microwave helps reduce the preheating

time but do take care not to overheat as it will take longer for the buffer to cool than it will to heat up.

Parallel DGGE Casting with gradient mixer

1. Secure the gradient mixer to a retort stand by using the red handle supplied and attach a 25cm piece of silicon tubing to the outlet port of the mixing chamber. Very carefully pass the tubing over to the PAGE insert and by using some masking tape fix the other end of it, connected to a syringe needle, between the glass plate cassettes. The needle should be positioned at the top of the gel in the middle of the glass plates. If using the optional but highly recommended MU-D01 peristaltic pump carefully thread the silicon tubing through the pump head before affixing the syringe needle to the glass plates, as described above. For polyacrylamide slab gels a flow rate of 5ml per minute (10 rpm) is sufficient.
2. After setting up the gradient mixer as stated above, place it on a magnetic stirrer by readjusting the height of the gradient mixer on the retort stand. Place a small magnetic stirring bar in the mixing chamber. Cleaver Scientific recommends the CSL-STIR and MU-S16 silicon tube (I.D. 1/8"). Please see the schematic diagram overleaf. However, if using gravity to fill the gel cassettes adjust the height of the gradient mixer so that it is above the PAGE insert.
3. Close the stopcock valves for the reservoir and mixing chambers.
4. Prepare the high and low denaturant concentration solutions in separate 50mL Falcon tubes, labelled 'HIGH' and 'LOW' respectively, as described in Parallel Gradient Gel Preparation.

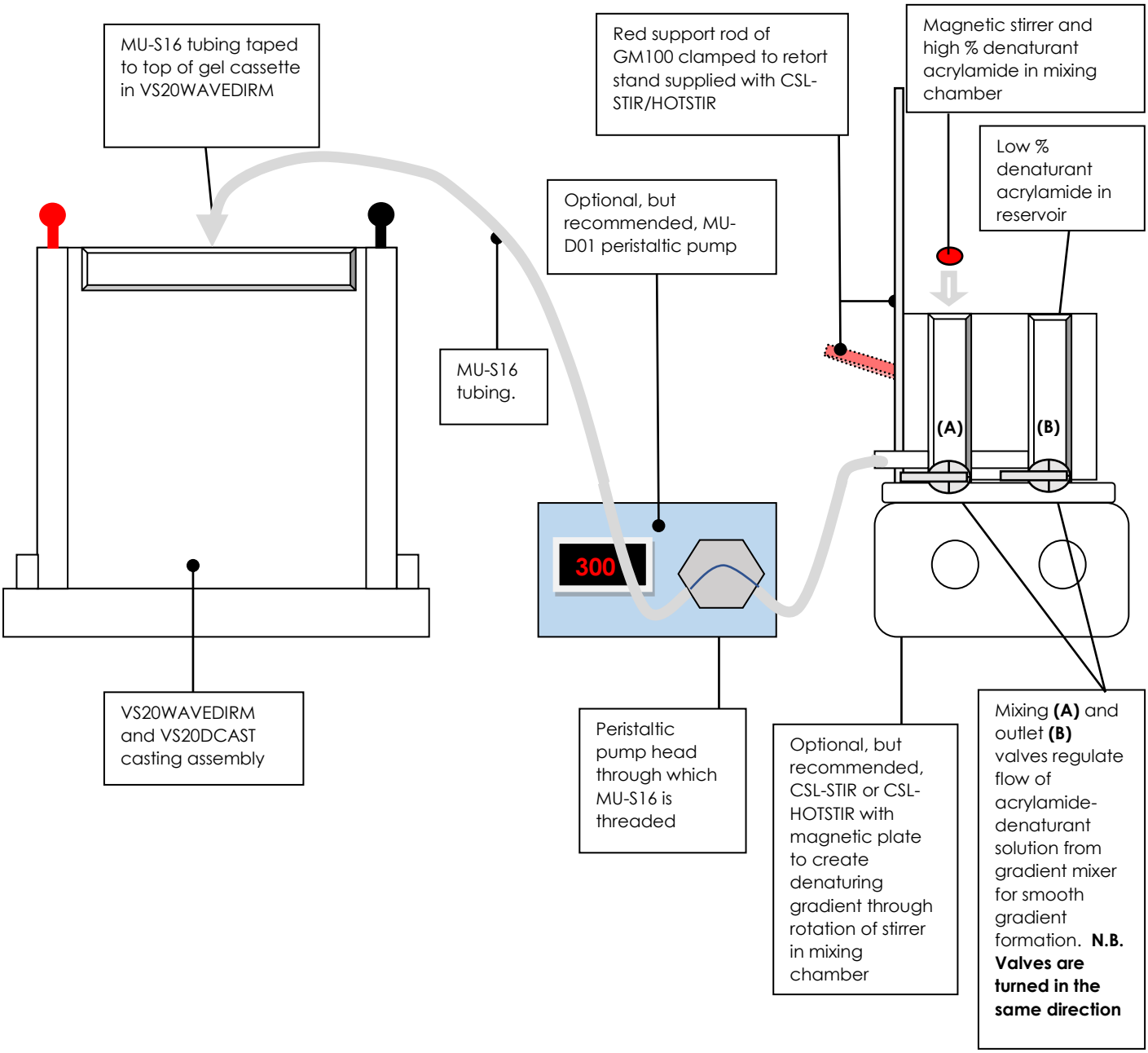
Optional: Add 100µl of Gradient Dye Solution per 5mL of high density solution to serve as a visual indicator of gradient gel formation.

DO NOT ADD POLYMERISING AGENTS AT THIS POINT UNTIL FIRST REVIEWING STEPS 5-12 BELOW.

5. BEFORE PROCEEDING WITH THE FOLLOWING STEPS, it is important to become familiar with the workings of the gradient mixer as described below, because once the polymerising agents are added to the acrylamide there is a maximum timeframe of 10 minutes to cast the gradient gel before polymerisation. A couple of trial runs with distilled water should be performed if necessary.

6. Add a final concentration of 0.09% (v/v) each of ammonium persulphate and TEMED solutions to the high and low percentage denaturant solutions. The 0.09% (v/v) concentrations allow no more than 10 minutes to finish casting the gel before polymerisation. Replace the cap and mix by inverting each tube gently several times.
7. Add the low percentage denaturant solution to the reservoir chamber and fill the stopcock valve with this solution by opening the valve, allowing the tube in it to fill before, again, closing the valve. PROCEED QUICKLY AND DO NOT ALLOW ACRYLAMIDE TO ENTER THE MIXING CHAMBER.
8. Now transfer the high percentage denaturant solution to the mixing chamber.
9. Start the magnetic stirrer.
10. Start the peristaltic pump and simultaneously open the outlet port of the mixing chamber of the gradient mixer. Open the connecting valve between the mixing and reservoir chambers.
11. Run the acrylamide denaturing gel mix slowly down from the upper centre of the gel cassette. Avoid any bubble formation, as bubbles inhibit polymerisation.
12. Allow all of the solution in the mixing chamber to empty before turning off the peristaltic pump.
13. Remove the tube from the glass cassette assembly. Insert the comb and allow the acrylamide solution to polymerise properly for 1 hour. Detach the gradient mixer from the retort stand and wash thoroughly in warm soapy water. Rinse with distilled water and drain before preparing a second gradient gel if desired.

Diagram of casting setup



Casting CDGE Gels

With the PAGE insert set up for casting, start making the appropriate denaturant gel solution following the directions below.

1. Add the required amounts of low density and high density solutions required for the desired denaturant percentage (see CDGE calculation) to a 50ml Falcon tube. Replace the cap and mix gently by inverting the tube.
2. Remove the cap and add a final concentration of 0.09% (v/v) each of ammonium persulphate and TEMED solutions to the denaturant solution. The 0.09% (v/v) concentrations allow no more than 10 minutes to finish casting the gel before polymerisation. Replace the cap again and mix by inverting each tube gently several times.
3. Insert a comb in the gel sandwich and tilt it so the teeth are at a slight angle (e.g. 20-30 degrees from the top left hand corner of the gel). This will prevent air from being trapped under the comb teeth when pouring the gel solution.
4. Pour or pipette the gel solution into the sandwich until the gel solution covers the wells of the comb. Straighten the comb to the desired well depth, by pushing it downwards at the angle between the glass plates. Add more gel solution if needed.
5. Allow the gel to polymerize for about 60 minutes. After polymerization, remove the comb by pulling it straight up, both slowly and gently.
6. Proceed to the section on Electrophoresis. Gel Running

1. Fit the lid and connect to a power supply.
2. Gels should be run at constant voltage, with voltage dependent on the size of the proteins undergoing separation. An initial setting of 100V with constant observation of migration and heat production is recommended. An exploratory study may be required to determine the optimal settings for individual proteins.

Running gels

Finalising Set Up

1. The electrophoresis tank should contain 7L of the appropriate running buffer.
2. When the running buffer has reached the desired temperature, switch off the VS20WAVE-DGGETC and disconnect the power cord. Remove the lid.
3. Gently remove the comb from each gel, taking care not to damage the wells. Detach the PAGE insert from the casting base by unlocking and turning the cam pins.
4. Transfer the PAGE insert to the VS20WAVE-DGGE tank, noting that the insert will only fit in one orientation.
5. Reconnect the power cord and turn back on the VS20WAVE-DGGETC. Wash the wells with running buffer to flush out any unpolymerised acrylamide or leached denaturants from the wells. If necessary, add more buffer to the "max fill" line of the electrophoresis tank. Return the lid to PAGE insert within the tank.
6. Allow the system to reach the set initial temperature before loading samples. This may take 10–15 minutes.

Sample Loading

Remove the lid and, having first ensured that the wells are free of any impurities, load the samples using a pipette and a sequencing loading tip. Be careful not to pierce the wells during sample delivery.

Running the gel

1. Attach the red and black power cables, which should be already screwed into the lid, to the corresponding ports within a suitable DC power supply. Cleaver Scientific recommends the POWERPRO500 programmable power supply or the nanoPAC-500.

2. Run the gel at 130 volts. Apply power to the VS20WAVE-DGGE and begin electrophoresis. As a precaution, always set the voltage, current, and power limits when possible.

N.B. The voltage should not exceed 180 V; otherwise electrophoretic heating may affect results.
Optional: If the power supply has a built-in timer, set the power supply timer to the desired run time, noting that the run time should be determined empirically for each fragment being analysed.

Removing the Gel

1. Following electrophoresis, turn off the power supply and temperature control unit. Disconnect the power cord and electrical leads and allow the heater to cool for approximately 1 minute in the buffer.
2. Gently remove the PAGE insert from the VS20WAVE tank, taking care to avoid contact with the heating element of the temperature control, which may be HOT!
3. Transfer the PAGE insert to a clean bench surface covered with paper towels. Twist the red and black screws on the PAGE insert to unlock the sliding clamps. Push the sliding clamps upwards to release the pressure on gel cassettes. Remove the gel cassettes and gently pry open the glass plates in the centre, using a spacer of the corresponding thickness or a gel-release tool.
4. If running two gels, cut the corner of one gel so it may be easily distinguished from the other and therefore identified.
5. Empty the buffer from the tank and rinse the heating element of temperature control unit with tap water followed by distilled water.

Staining and Photographing gels

1. Carefully tease the gel, using either a spacer or a spatula, from the glass plate to which has attached, into a suitable dish or tub containing 250 ml of running buffer and 25 µl of 10 mg/ml ethidium bromide (50 µg/ml).
2. Stain for 5–15 minutes.
3. After staining, carefully transfer the gel into a dish containing 250 ml of 1x running buffer.
4. Destain for 5–20 minutes.
5. Place the gel on a UV transilluminator of a suitable gel documentation system and photograph (the Cleaver Scientific microDOC [CSL-MDOCUVTS312] and omniDOC [CSL-OMNIDOC], both with 312nm transilluminators, are ideal for this purpose). 1D image analysis with band pattern matching within individual gels may be performed using CLIQS 1D, while CLIQS 1D Pro may be used for band pattern matching between different gels.

Troubleshooting

Equipment Troubleshooting

Problem	Cause	Solution
Temperature Controller		
No display with power on	Burned out fuse or inner circuitry	Do not attempt to open housing as warranty will be immediately invalidated. Contact your distributor or manufacturer
Display shows "Cut"	Over-temperature cut-out has operated	Check the set temperature is correct and that the over-temperature cut-out temperature is set at least 5°C above the set temperature.
Temperature does not rise when expected	Set temp is lower than liquid temp Set temperature is too close to ambient	Check that the bath set temperature is correct Increase the set temperature or fit accessory cooling e.g. cool pack
Temp continues to rise when not expected	Set temp is higher than liquid temp	Check that the DGGE controller set temperature is correct
Alarm light on	Over-temperature cut-out has operated Low liquid level float switch has operated	Check the over-temperature cut-out is set appropriately Check that the liquid level in the WAVE-DGGE tank is adequate
Display shows "Flot"	Liquid level has dropped below minimum level	Check that the liquid level in the WAVE-DGGE tank is adequate
Display shows "–Al-"	High temperature warning alarm has tripped	Check that the set temperature is correct Check that the setting for the high temperature alarm is correct Check that the liquid level in the WAVE-DGGGE tank is adequate
Casting Gels		
Leaking during gel casting	Incorrect assembly of gel sandwich	Check that the spacers and glass plates are flush before pouring acrylamide; check with a small volume of water beforehand
	Over-turned cams	Cams should not be turned more than 90 degrees in alternate directions

	PAGE insert, and glass plates assembled on casting base	All perform glass plate assembly on flat surface, before transferring to casting base; never on the casting base
	Chipped glass plates	Check bottom of glass plates for chips; replace if necessary

Application Troubleshooting

Parallel DGGE	
Normal and mutant DNA unresolved	1. Perform time course 2. Recalculate gradient range or run a time course gel
Air bubbles in gel	• Clean plates
Fuzzy DNA bands	• Clean wells before use; check for matching comb and spacer thickness; increase polymerisation time
Bands do not migrate far enough into gel	1. Increase run time 2. Decrease acrylamide concentration 3. Decrease denaturant concentration
DNA leaks between wells	1. Acrylamide not polymerised; add more APS and TEMED (0.1% final conc.) 2. Degas acrylamide before casting 3. Allow to polymerise over 60' 4. Do not overload sample wells; reduce sample volume
Skewed or distorted bands	1. Impurities in acrylamide; filter or check shelf life date 2. Carefully load DNA samples; do not puncture wells
CDGE	
Normal and mutant DNA unresolved	Repeat parallel DGGE to recalculate constant denaturant concentration
Air bubbles in gel	• Clean plates
Fuzzy DNA bands	• Clean wells before use; check for matching comb and spacer thickness; increase polymerisation time
Bands do not migrate far enough into gel	1. Increase run time 2. Check and if necessary decrease acrylamide concentration 3. Re-check denaturant concentration and decrease if necessary
DNA leaks between wells	1. Acrylamide not polymerised; add more APS and TEMED (0.1% final conc.) 2. Degas acrylamide before casting 3. Allow to polymerise over 60' 4. Do not overload sample wells; reduce sample volume
Skewed or distorted bands	1. Impurities in acrylamide; filter or check shelf life date 2. Carefully load DNA samples; do not puncture wells

Care and Maintenance

Cleaning Vertical Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60°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 pyro carbonate) 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.

Ordering information

VS20WAVE-DGGE	Complete Denaturing Gradient Gel Electrophoresis System, 20x20cm; includes: temperature control unit, cam casting base, glass plates with 1mm bonded spacers, 2x 24-sample combs and gradient mixer – 240 VAC version
VS20WAVE-DGGE$	VS20WAVE-DGGE – 110VAC version
VS20WAVE-DGGETC	VS20WAVE-DGGE Temperature Control Unit – 240VAC version
VS20WAVE-DGGETC$	VS20WAVE-DGGETC – 110VAC version
GM100	Gradient Mixer, 100mlAccesso
CSL-STIR	CSL Magnetic Stirrer, 19x19cm
MU-D01	Single Peristaltic Pump
MU-S16	Silicon tube I.D. 1/8", 25 ft, for MU-D01
POWERPRO500	PowerPRO Power Supply, 500V, 800mA, 300W
CLIQS 1D	1D image analysis with band pattern matching
CLIQS 1D PRO	1D image analysis with band pattern matching between different gels DGGE Package
VS20WAVE-DGGEKIT	VS20WAVE Package Deal; includes: VS20WAVE-DGGE, CSL-STIR, MU-D01, MU-S16, CS-500V – 240 VAC version
VS20WAVE-DGGEKIT$	VS20WAVE-DGGEKIT – 110 VAC version

Warranty

The Cleaver Scientific Ltd. (CSL) Vertical Electrophoresis units have a warranty against manufacturing and material faults of twelve months from date of customer receipt.

If any defects occur during this warranty period, 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 Cleaver Scientific Ltd:

Cleaver Scientific Ltd.

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Rugby, Warwickshire, CV22 7DH

Tel: +44 (0)1788 565300

Email: info@cleaverscientific.com