



pHaser Isoelectric Focusing System

For Running Immobilized pH Gradient Strips





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Good luck with your research and do not hesitate to contact us should you have any questions.



OVERVIEW

Introduction

Two-dimensional gel electrophoresis is a high resolution technique for separating complex mixtures of proteins. Traditionally, in 2-D electrophoresis, the isoelectric focusing (IEF) 1-D separation was performed in tube gels with carrier ampholytes. An alternative to IEF with carrier ampholytes utilizes immobilized pH gradient gels in which ampholytes are covalently linked to acrylamide and a pH gradient is fixed in the gel.

Immobilized pH gradient strips are easy to use, less sensitive to protein load and contaminants, and offer excellent resolution for soluble proteins. The pHaser (Figure 1) was developed for isoelectric focusing of immobilized pH gradient strips (11, 17, 18 cm) with the Investigator™ 2-D Gel Electrophoresis System.

Genomic Solutions' immobilized pH gradient strips are known as pHlash strips.

Components of the pHaser Kit

- pHaser housing (Figure 2)
- pHaser trays for 11 cm and 18 cm gels (Figure 2)
- wicks, 100 per pack
- non-conducting oil, 50 ml
- Urea/thiourea Rehydration/Solubilization Buffer (10 X 1 ml)
- Equilibration Buffer I (4 x 50 ml)
- Equilibration Buffer II (2 x 50 ml)
- Equilibration trays, 10 per pack

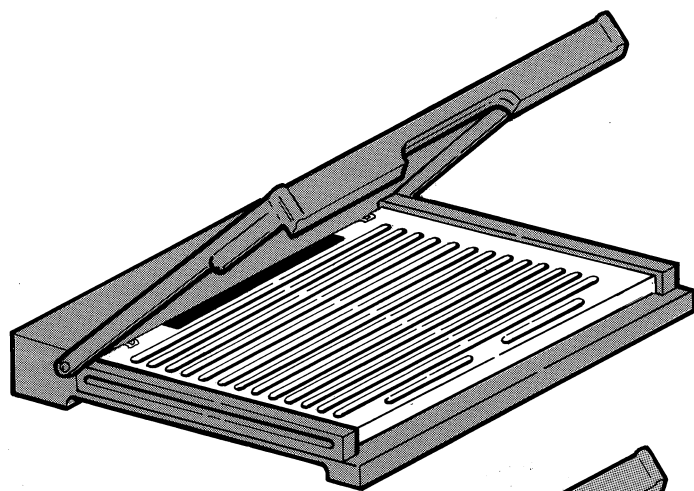


Figure 1. pHaser™ Running Device

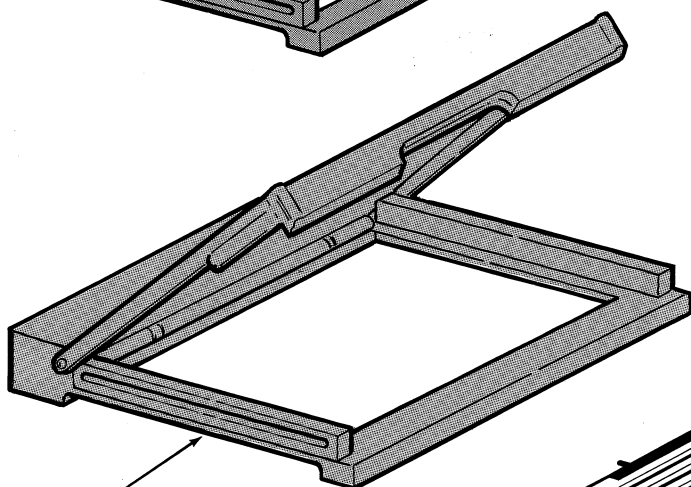


Figure 2. pHaser Components



pHLASH STRIP REHYDRATION, FOCUSING AND EQUILIBRATION

Rehydration

NOTE: Try to avoid salt concentration above 20 mM. Wear gloves while handling the strips. The following protocol pertains to 18 cm pHlash strips.

1. Combine up to 1 mg of sample protein with desired Solubilization/ Rehydration Buffer in a total volume of 400 μ l.
2. Choose a running tray to place into the pHaser. If you wish to run 18 cm pHlash strips, use the tray with the larger gap between electrodes. If you wish to run 11 cm strips, choose the tray with the smaller gap between the electrodes and use the parameters supplied with the strips.
3. Evenly distribute sample mixture between electrodes in pHaser tray.
4. Carefully peel off the protective film from the acidic end of a pHlash strip.

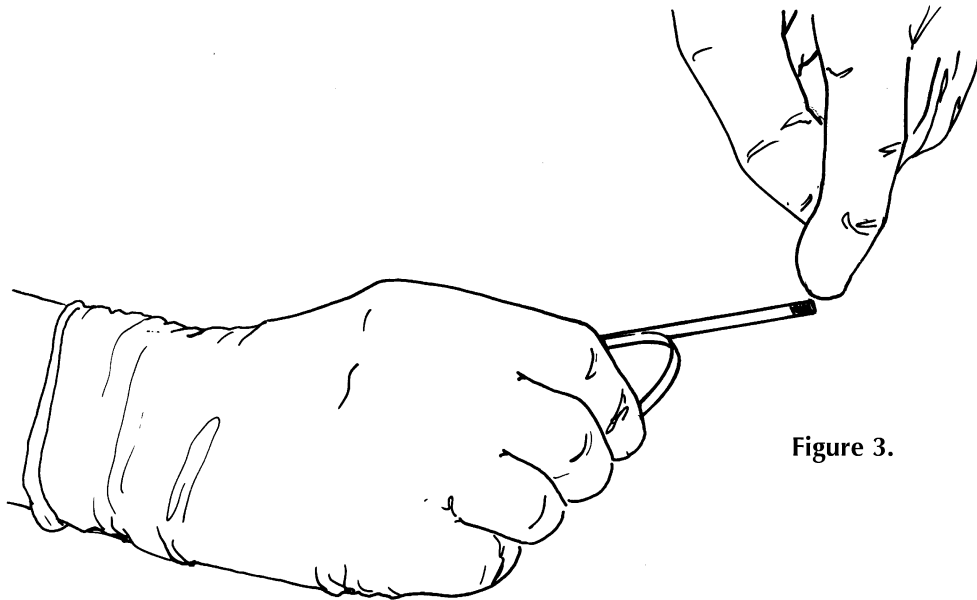


Figure 3.

NOTE: The orange tape is applied to the back of the pHlash strip. To find the gel face, inspect the strip for the raised edge of the tape; the gel is on the reverse side.

5. Place the pHlash strip gel side down onto the sample mixture with the anodic (colored) end toward the anode (arrow-shaped end of the groove in the tray). Make sure the strip makes contact with the solution along its entire length, and that no bubbles are trapped under the strip.
6. Put pHaser tray in an airtight bag with a moist piece of filter paper and rehydrate for a minimum of 16 hours.



Focusing

NOTE: If the pHaser lid is lifted, the power will be turned off.

1. Slide tray into pHaser housing and make sure plugs are fully engaged.
2. Place the pHaser on top of the Investigator Chiller plate.
3. Set the temperature on the Investigator™ Chiller so that the temperature strip on the tray reads 15-20°C .
4. Place a wick moistened slightly with 18 MOhm water under each end of the pHlash strip between the gel and electrode.

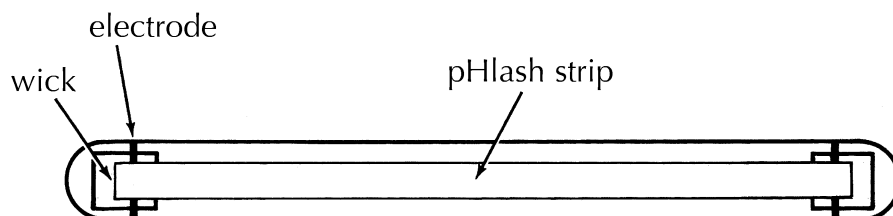


Figure 4. Placing the wicks under the pHlash strip.

5. Pour a small amount of mineral oil (approximately 2 ml) over the strip to cover it. Do not overfill the groove with oil.
6. Close the pHaser cover and connect it to the Investigator power supply.

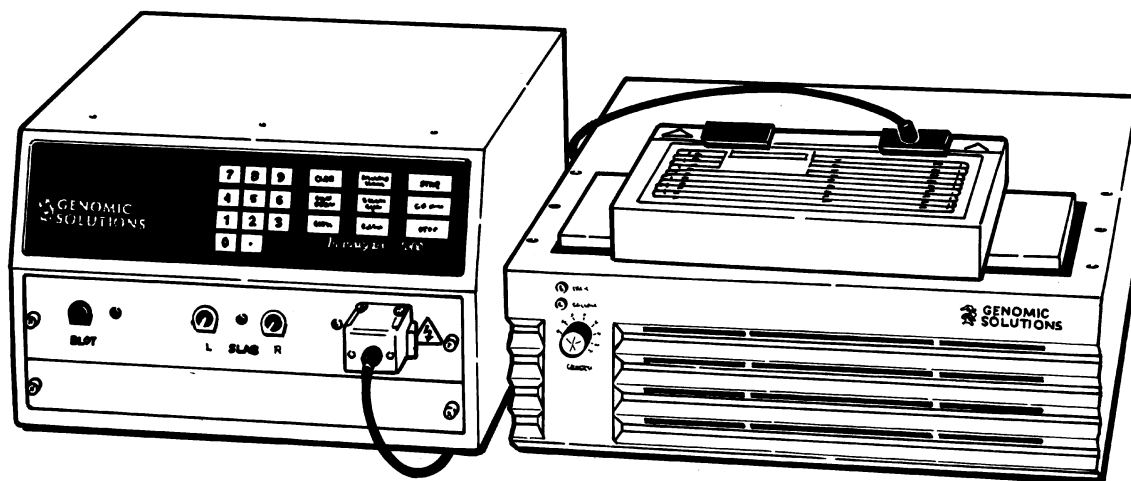


Figure 5.

7. Focus the strips using the following parameters. These parameters are for 18 cm strips.

Focusing the Gels

Action	Display
Press FUNCTION SETUP	PROCESS (1P, 2F, 3S, 4B)
Press 2 ENTER	# OF GELS: xx
Enter the number of pHlash strips you are running in the experiment. ENTER (The max number is 10).	MAX VOLTAGE (V): XXXX
Press 5000 ENTER to set the maximum voltage for pHlash strips to 5000 Volts.	Vhold (holding voltage): XXX
Press 125 ENTER to set the holding voltage to 125.	DURATION (h 0-99): XX
Enter the number of hours for the duration of your run. 24 is recommended. Press 24, ENTER	DURATION m (0-59): XX
Enter the number of minutes (in addition to the hours already entered) for the duration of your run. 30 minutes are recommended. Example: Press 30 ENTER.	MAX CURRENT (μ A): XXX
Set the current per gel. 80 is the max μ A/gel. Press ENTER. The power supply will determine the total current.	VOLT HOURS: XXXX
Press 100,000 ENTER to set focusing to 100,000 Volt-hours.	

NOTE: For reproducible patterns, the Volt-hours must be the same from run to run.

Checking your program

Action	Display
Press FUNCTION SET UP	PROCESS (1P, 2F, 3S, 4B)
Press 2 to select focusing.	
Press ENTER to check your previous entry into the focusing program, starting with # OF GELS. The previously entered value will show on the first line of the screen.	
Press ENTER again to check the MAX VOLTAGE.	
Repeat for Vhold, DURATION, MAX CURRENT and VOLT HOURS.	

Starting your program

Press START then 2.

NOTE: If you need to stop the IEF run, press STOP and perform necessary tasks such as changing the wicks. In some laboratories, if crude samples are being processed, wicks are changed several times during focusing to remove contaminants which have accumulated. To restart the power supply, press CONTINUE then 2. If the power supply has been stopped, the time is "remembered" by the power supply but the volt-hours are reset to zero. It is best to record the accumulated volt-hours before stopping.

Monitoring a Focus Run

Note: Monitoring a program is used if simultaneously running the slab gel and IEF functions.

Action	Display
Press REVIEW.	PROCESS (1P, 2F, 3S, 4B)
Press 2.	The actual reading for prefocusing are displayed and will be updated once per second throughout the run.
Press ENTER to leave the REVIEW mode.	The time of day.

Finishing the Run

NOTE: When focusing is complete, an alarm will sound. This indicates that the designated time has elapsed. The display will read "PRESS (.) to disable the beeping sound". Once the beeping has been disabled, a holding voltage of 125 V will be maintained.

Caution: If the alarm sounds and final volt-hours have not been reached, an error in programming may have occurred. Press REVIEW after the process is complete to check focusing parameters.

Action	Display
Press "." to stop beeping.	
Press STOP.	PROCESS (1P, 2F, 3S, 4B)
Press 2 to stop focusing and turn off the power supply.	

NOTE: If the power fails when a process is running, the supply will restart when power is restored. The screen will display:

!!POWER OUTAGE!!
@XX:XX for YY:YY:YY

The variable "XX:XX" is the time of day the power failed, and the "YY:YY:YY" is the total period for which power was out. This screen can be cleared by pressing the ENTER key.

- When focusing is complete, the strips can be frozen at -70°C, or equilibrated and used immediately.

Equilibration

1. When focusing is complete, disconnect the pHaser from the power supply and carefully open the cover.
2. Using forceps, grasp the end of a strip and lift it out of the groove (Figure 6).
3. Place the strip in an equilibration tray.
4. Pour 10 ml of Equilibration Buffer I into the tray and incubate strip while gently shaking for 10 minutes. Discard buffer and repeat step 4.
5. Pour off the buffer, and pour in 10 ml of Equilibration Buffer II. Incubate while gently shaking for 10 minutes.
6. Strips are now ready to be run on the second dimension gels. Refer to page 6 in the 2-D Casting and Running Manual.

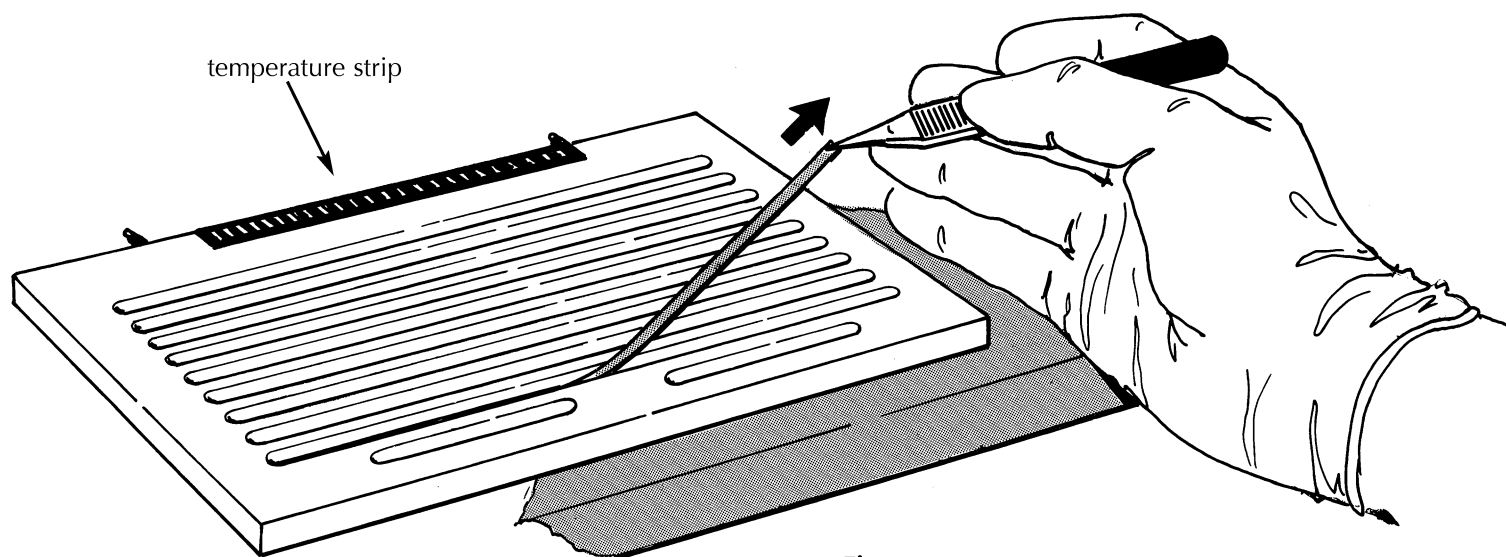


Figure 6.

BUFFERS

Solubilization & Rehydration Buffers

0070-3981 Urea Solubilization/Rehydration Buffer

Components

Reagent	Amount	Final Concentration
Urea	48 g	8 M
CHAPS	2 g	2%
DTT	150 mg	10 mM
Ampholytes (pH 3-10)	2 ml	2%
Bromphenol Blue	10 mg	0.01%
18 MOhm Water	to 100 ml	

Procedure

1. Add 50 ml of 18 MOhm water to a 100 ml graduated cylinder.
2. Place the cylinder on a magnetic stirrer.
3. Slowly add the urea to the water while stirring.
4. When all the urea has dissolved, add the other ingredients and stir until everything has dissolved.
5. Remove the magnetic stirring bar and adjust the volume to exactly 100 ml with 18 MOhm water.
6. Filter the solution through a 0.45 micron filter.
7. Store frozen at -70°C in 1 ml aliquots.

Note: If there is a problem dissolving the urea, you may slightly warm the solution to 30°C. Exceeding this temperature may result in carbamylation of proteins.

This solution is used to solubilize the protein samples and act as a vehicle for their transfer to the pHlash strip.

0070-4019 Urea/Thiourea Solubilization/Rehydration Buffer

Components

Reagent	Amount	Final Concentration
Urea	42 g	7 M
Thiourea	15.2 g	2 M
CHAPS	2 g	2%
DTT	1.0 g	65 mM
Zwittergent	1.0 g	1%
Bromphenol Blue	10 mg	.01%
Ampholytes (pH 3-10)	800 µl	0.80%
18 MOhm Water	to 100 ml	



Procedure

1. Add 50 ml of 18 MOhm water to a 100 ml graduated cylinder.
2. Place the cylinder on a magnetic stirrer.
3. Slowly add the urea to the water while stirring.
4. When all the urea has dissolved, add the thiourea and stir well until dissolved.
5. Add the other ingredients and stir until everything has dissolved.
6. Remove the magnetic stirring bar and adjust the volume to exactly 100 ml with 18 MOhm water.
7. Filter the solution through a 0.45 micron filter.
8. Store frozen at -70°C in 1 ml aliquots.

Note: If there is a problem dissolving the urea, you may slightly warm the solution to 30°C.

This product is supplied frozen and should remain frozen until use. The concentration of Zwittergent® in the buffer is near its maximum solubility in the presence of high concentrations of urea; therefore, the buffer must be thawed at 30° C in order to re-dissolve all of its constituents. If all of the constituents are not dissolved, the solubilization of proteins in the samples will be less than optimal.

Equilibration Buffers

0080-0233 Equilibration Buffer I

Components

Reagent	Amount	Final Concentration
Urea	360 g	6 M
DTT	20 g	130 mM
Glycerol	300 ml	30%
Tris base	5.44 g	45 mM
SDS	16 g	1.6%
Bromphenol Blue	20 mg	0.002%
Acetic acid, glacial	~15 ml	to pH 7
18 MOhm Water	to 1 L	

Procedure

1. Add 250 ml of 18 MOhm water and 300 ml of glycerol to a 1 L graduated cylinder.
2. Place the cylinder on a magnetic stirrer and stir until glycerol and water are mixed.
3. Slowly add the urea to the water/glycerol solution while stirring.
4. When all the urea has dissolved, add the other ingredients except glacial acetic acid and stir until everything has dissolved.

- Using a pH meter, adjust the pH of the solution to 7.0 with the glacial acetic acid. It will take approximately 15 ml.
- Remove the magnetic stirring bar and adjust the volume to exactly 1 L with 18 MOhm water.
- Store frozen at -70°C in 50 ml aliquots.

Note: This buffer is used for equilibration of 1-D gels prior to loading onto the 2-D gel. It coats the separated proteins with SDS and reduces sulfhydryl groups. This recipe makes one liter of solution. Aliquot into 50 ml tubes and freeze until needed.

**0080-0232 Equilibration Buffer II
Components**

Reagent	Amount	Final Concentration
Urea	360 g	6 M
Iodoacetamide	25 g	135 mM
Glycerol	300 ml	30%
Tris base	5.44 g	45 mM
SDS	16 g	1.6%
Bromphenol Blue	20 mg	0.002%
Acetic acid, glacial	~15 ml	to pH 7
18 MOhm Water	to 1 L	

Procedure

- Add 250 ml of 18 MOhm water and 300 ml of glycerol to a 1 L graduated cylinder.
- Place the cylinder on a magnetic stirrer and stir until glycerol has dissolved.
- Slowly add the urea to the water/glycerol solution while stirring.
- When all the urea has dissolved, add the other ingredients except glacial acetic acid and stir until everything has dissolved.
- Using a pH meter, adjust the pH of the solution to 7.0 with the glacial acetic acid. It will take approximately 15 ml.
- Remove the magnetic stirring bar and adjust the volume to exactly 1 L with 18 MOhm water.
- Store frozen at -70°C in 50 ml aliquots.

Note: This buffer is used for equilibration of IEF gels prior to loading onto the 2-D gel. It coats the separated proteins with SDS and alkylates the reduced sulfhydryl groups. This is especially important if one will use mass spectrometry for protein characterization. This recipe makes one liter of solution. Aliquot into 50 ml tubes and freeze until needed.



PARTS LIST

Immobilized pH Gradient (IPG) Running System (IPG001)

	Part No.
pHaser	0070-3537
Flat Bed Running System	0070-4021
Equilibration Trays (5 per pack) (2 packs per kit)	0080-0045
Manual	0070-4017

pHaser Chemical Kit	0070-3546
Equilibration Buffer I, 50 ml, (quantity of 4 in kit)	0080-0233
Equilibration Buffer II, 50 ml (quantity of 2 in kit)	0080-0232
Wicks (100 per pack)	0070-3978
Non-Conducting Oil for IPG (50 ml)	0070-3977
Urea/Thiourea Rehydration/Solubilization Buffer, 10 x 1 ml	0070-4019
3-10 pHlash Strips (10 per pack)	0070-4313

Additional Buffer

Urea Rehydration Buffer, 10 x 1 ml	0070-3981
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pHlash Strips (Immobilized pH Gradient Strips)

pH 3-10, 18 cm (10 per pack)	0070-4313
pH 4-8, 18 cm (10 per pack)	0070-4318
pH 3-5, 18 cm (10 per pack)	0070-4314
pH 5-7, 18 cm (10 per pack)	0070-4315
pH 4-5, 18 cm (10 per pack)	0080-0324
pH 5-6, 18 cm (10 per pack)	0080-0325
pH 6-7, 18 cm (10 per pack)	0080-0326
pH 7-11, 18 cm (10 per pack)	0070-4316