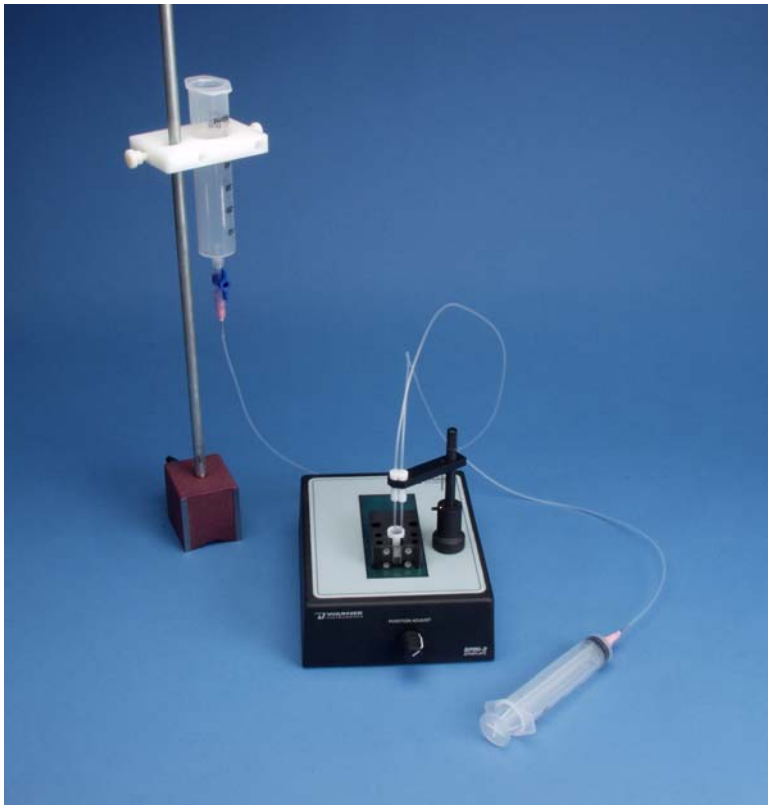
Bilayer Perfusion System
Model BPS-2

WARNER INSTRUMENTS
A Harvard Apparatus Company



Warner Instruments • 125 Dixwell Avenue, Hamden, CT 06514
(800) 599-4203 / (203) 776-0664 • (203) 776-1278 – fax
www.warneronline.com

TABLE OF CONTENTS

Thank you for purchasing the **BPS-2 Planar Lipid Bilayer Perfusion System** from Warner Instruments. This simple to use apparatus will allow you to easily exchange solutions on either side of your bilayer chamber.

The **BPS-2** contains all the components necessary for the assembly of a basic bilayer perfusion system.

Features include:

- Dedicated design for bilayer applications
- System completely contained within Faraday cage
- Simple, manual operation
- Quiet, gravity feed of incoming solution

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED FOR USE ON HUMAN SUBJECTS

NOMENCLATURE

Text conventions

To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instrument product numbers are presented using **bold type**.
- References to separate components are specified using UNDERLINED SMALL CAPS.
- References to specific controls are specified using NON-UNDERLINED SMALL CAPS.
- References to control settings are specified in *italic type*.
- Special comments and warnings are presented in highlighted text.
- Any other formatting should be apparent from context.

INTRODUCTION

Exchanging solutions (termed perfusion) normally occurs following incorporation of a channel into the bilayer membrane, or when experimental conditions require an alteration in ionic conditions or the removal of a previously added compound. Ideally, a good perfusion system is capable of replacing the solution in a bilayer chamber without interrupting the recording process or rupturing the membrane. However, the large amplitude noise, both mechanical and electrical, makes such an attempt likely to result in a broken membrane.

Solution exchange in a bilayer chamber has traditionally been achieved using a home-made device. While many techniques have been used, the most common design incorporates a gravity feed platform. Other approaches include pump driven, siphon driven, or manually-applied pressure systems.

Most bilayer perfusion systems are comprised of three components: a solution input pathway, a 'perfusion head', and a solution removal pathway. Solution is introduced into the bilayer chamber via the input pathway and is extracted via the removal pathway. The perfusion head is dedicated to positioning and holding the input and removal perfusion lines. In general, fresh solution is added to the bottom of the recording chamber while the perfusate is removed from the top.

The primary goal of a perfusion system is to deliver fresh solution to the chamber without breaking the bilayer membrane. Generally speaking, membrane breakage occurs as a result of excessive mechanical or electrical effects. Mechanical effects include turbulences associated with flowing fluids and hammer effects associated with the action of a pump or with an aspirator (input path and removal path, respectively). Electrical effects include loss of shielding, introduction of charge, and a large increase in system capacitance when the flow lines are placed into contact with the chamber bath.

Since the solution input pathway traffics solution from a volume reservoir to the chamber being perfused, and since solution flow is one cause of membrane breakage, the BPS-2 employs a gravity driven mechanism to deliver solution to the chamber. This approach is viable because the resulting solution flow is both smooth and even, and because the design is simple to implement and maintain. Removal of solution in the BPS-2 is achieved by aspiration. This system uses a manual, syringe driven aspirator which can be easily modified to accommodate vacuum driven aspiration, if desired.

The perfusion head represents the mechanical heart of the system and is dedicated towards providing a stable, reproducible mechanism for holding, inserting and removing perfusion lines in the bilayer chamber. The use of a perfusion head for solution exchange can greatly increase the rate of successful perfusion without membrane breakage.

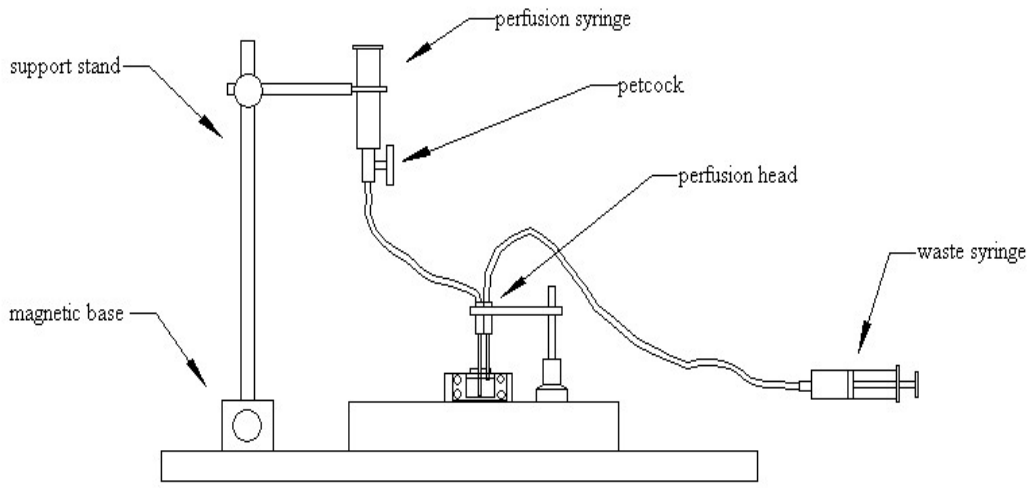
EQUIPMENT

The **BPS-2** includes:

Item	Qty shipped
Perfusion head assembly	1
Small Allen wrench	1
Medium Allen wrench	2
Small steel plate	1
MBB magnetic base	1
19” support rod	1
MSH/60M modular syringe holder	1
30 cc and 60 cc syringes	1 ea
PE-160 tubing	10 ft
G150-4 glass capillary tubes	225 pk
1/16” ID C-Flex tubing, 5/8” length	6 ea
Stopcock valve for syringe	1
18 GA blunt end needles	2

SETUP

The set up is straightforward. First we will assemble and position the perfusion head adjacent to the bilayer chamber. Next, we will set up the perfusion syringe and run a line from the syringe to the input side of the perfusion head. Finally, we will assemble and connect the aspiration side to the perfusion head. Waste is drawn off using the waste syringe, or alternatively, using a vacuum driven aspirator. Refer to the drawing below for illustration.

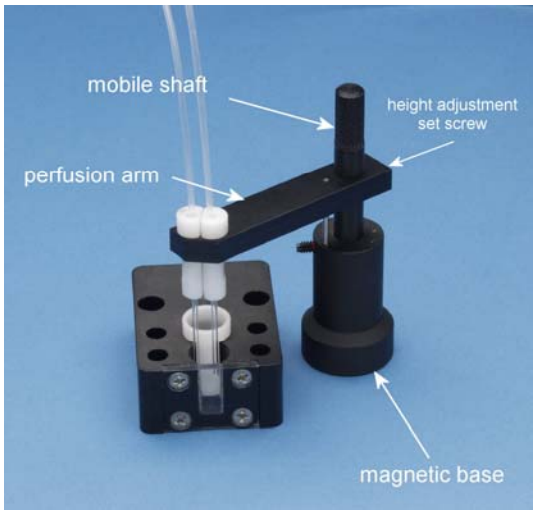


Note: In the following assembly instructions, it is important for the input side (reservoir and line) of the perfusion system to remain inside the Faraday cage since the conducting solutions within these components can act as radio frequency (RF) leaks. The requirement for a vacuum aspiration path to be contained entirely within the cage is less stringent since the aspirator generally keeps the line clear of conducting fluids when not in use.

Assemble the perfusion head

The perfusion head comes pre-assembled. All that is needed is to adjust the position of the perfusion arm and to insert the capillaries and perfusion lines.

Examine the perfusion head. Note that it is comprised of a PERFUSION ARM fixed to a MOBILE SHAFT and a MAGNETIC BASE. The magnetic base allows easy attachment of the perfusion head onto the top of the **SPIN-2** bilayer stirplate, or it can be placed onto a non-magnetic surface using the included small steel plate. The steel plate serves to provide additional stability to the base.



As noted before, the perfusion arm is fixed to a mobile shaft. The shaft moves up and down, and rotates to the right in the up position. This motion will serve to quickly and easily move the perfusion lines in and out of the bilayer chamber when needed. There is a small white thumbscrew on the top-rear of the magnetic base for adjusting the tension of the mobile shaft.

Adjust the perfusion head

1. Begin by loosening the small set screw (HEIGHT ADJUSTMENT SET SCREW) on the back of the perfusion arm. A small Allen wrench is provided for this purpose.
2. Place a bilayer chamber (with cup) on a flat surface and position the perfusion head next to it as shown in the figure to the right.
3. Push the mobile shaft all the way into its down position and move the loosened perfusion arm such that the bottom of the white inserts within the perfusion arm are approximately 0.5” above the chamber well. See the image above for proper positioning of the arm over the chamber.
4. Tighten the HEIGHT ADJUSTMENT SET SCREW so it gently holds the perfusion arm into place. A subsequent adjustment may be necessary before final tightening.
5. Test the motion of the perfusion head up and down, and left to right. Make adjustments to the height and tension as necessary.

Insert the capillaries

The next step is to place two capillaries into the perfusion head. A single capillary is placed into the bottom of each white receptacle on the perfusion arm. These white receptacles are factory loaded with a small section of C-Flex tubing, it is into this C-Flex tubing that you will insert your capillaries and perfusion tubing. In general, the rear capillary acts as the solution input channel and the front capillary will be the solution removal channel.

6. Pick the perfusion head up and adjust each white receptacle so it is centered within it's travel range.

   Note: There is a small set screw (on the side of the perfusion arm) for each white receptacle. These set screws can be loosened and tightened to release and hold the white receptacles in place.

7. Remove a glass capillary from its container and break it into two pieces approximately 2.5 and 3.5 cm long.

   Note: Avoid cutting yourself!

8. Carefully place the longer capillary piece into the rear white receptacle in the perfusion arm. Insert the broken end of the capillary into the C-flex tubing far enough to make a good seal, approximately 2-3 mm.

   Note: The relative position of the capillaries in the photo on page 7 is incorrect. The image shows the white receptacles *side-by-side*. They should be *front-to-back*.

9. Carefully place the shorter capillary into the front white receptacle in the perfusion arm such that the bottom of the rear capillary is approximately 1 cm lower than the bottom of the front capillary.

Adjust the capillary heights

10. Replace the perfusion head next to the bilayer cup and chamber as in step 3.

11. Using the HEIGHT ADJUSTMENT SET SCREW, adjust the height of the perfusion arm so that the longer (rear) capillary tube is approx 1-2 mm above the bottom of the chamber. Tighten the HEIGHT ADJUSTMENT SET SCREW.

    Note: This step is to allow room for your stirbars.

12. Place a working volume of water into your chamber and adjust the height of the shorter (front) capillary tube so that it just touches the surface of the solution. This is your aspiration height.

    Note: This height adjustment of the aspiration capillary is achieved by loosening the associated set screw on the side of the perfusion arm and moving the white receptacle up or down as needed.

Assemble the perfusion reservoir

13. Attach the 19" threaded rod to the MBB magnetic base. Place this support stand in the left rear corner of your Faraday cage and attach the MSH/60M modular syringe holder to the top of the rod.
14. Remove the plunger from the 60 cc syringe. Attach a stopcock valve to the syringe and attach a Luer-to-tubing connector to the valve. Mount the syringe assembly to the modular syringe holder.
15. Cut and run a length of PE tubing (1-2 ft) from the BLUNT-END NEEDLE on the syringe to the *input* white receptacle on the perfusion arm. The *input* white receptacle will be the rear or lower capillary tube.
16. Cut the non-syringe end of the input PE tubing on an angle and push it into the C-Flex tubing in the top of the input white receptacle. This will establish the solution input path of the perfusion system.

Assemble the perfusion waste syring

17. Attach a blunt-end needle to the 30 cc syringe. Exercise the plunger but leave it in place.
18. Cut and run a length of PE tubing (2-3 ft) from the BLUNT-END NEEDLE on the 30 cc syringe to the *output* white receptacle on the perfusion arm. The *output* white receptacle will be the front or higher capillary tube.
19. Cut the non-syringe end of the output PE tubing on an angle and push it into the C-Flex tubing in the top of the output white receptacle. This will establish the solution output path of the perfusion system.

And finally

20. Close the stopcock and fill the reservoir with solution.
21. Place a paper towel under the input (lower) capillary tube and open the stopcock. Allow sufficient solution to flow to completely fill the flow line and tube. Assure yourself there are no bubbles in the line as they will kill your membrane.

OPERATION

This perfusion system is structured so that you add solution to the bottom of the cup and draw waste off from the top. During solution exchange, the first activity is to place the perfusion tubes into the cup (or chamber) and begin solution flow by opening the stopcock. Hopefully this step occurs quickly and without breaking the membrane. Inflowing solution gently fills the chamber from the bottom and waste is removed from the top. The rate of waste removal is achieved by manipulation of the waste syringe.

When not actively perfusing, the perfusion head can be held directly above the chamber for rapid deployment. You may also keep it to the side to facilitate easy access to the chamber.

Operation is direct and simple. The input perfusion tube is loaded (primed) with solution and the perfusion arm is placed at the ready above your bilayer chamber. When you are ready to exchange solution the perfusion arm in quickly moved into position and solution flow initiated. Once sufficient solution has been exchanged, the perfusion arm is swung up and out of the way.

A couple of caveats:

1. Make sure your input perfusion line is completely full of solution before placing it into your cup. Any bubbles introduced will certainly break your membrane.
2. Have the WASTE SYRINGE ready to accept solution before opening the stopcock. In other words, have the WASTE SYRINGE empty and in hand first.
3. It is relatively simple to move the plunger out on the WASTE SYRINGE using your thumb as the new solution flows into the cup.
4. Start out by just changing solutions with no membrane present until you get your timings and flow rates dialed in. Once you get the hang of this system, you'll find that its very easy to use and reliable exchanges rates on the order of 5-10 ml/min are achievable.

APPENDIX

Warranty and service

Warranty

The **BPS-2** is covered by our Warranty to be free from defects in materials and workmanship for a period of 1 year from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse. If required, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments.

Service

We recommend that all questions regarding service be referred to our Technical Support Department.

- Normal business hours are 9:00 AM to 5:00 PM (EST), Monday through Friday.
- Our offices are located at 1125 Dixwell Avenue, Hamden, CT 06514.
- We can be reached by phone at (800) 599-4203 or (203) 776-0664. Our fax number is (203) 776-1278.
- We can be reached by e-mail at support@warneronline.com or through the web at http://www.warneronline.com.