BioNeb®

Cell Disruption System*

Instruction Manual

* U.S. patent number 5506100
International patents issued and pending

BioNeb® Cell Disruption System
Instruction Manual Version 3.0, April, 2000

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Cell Disruption

Introduction

Principle of Nebulization

Breaking living cells, isolating cell organelles, and fragmenting large molecules are three standard procedures used daily in research and industrial laboratories.

The **BioNeb® Cell Disruption System** is an instrument that nebulizes liquid (i.e., reduces liquid to a fine spray) to generate uniform shearing forces that can break cells and molecules. In the process of droplet formation, large molecules or cells suspended in the liquid being nebulized are forcefully distributed from the liquid into the forming droplet. This creates a transient laminar flow in the microcapillary channel formed between the surface of the liquid and the forming droplet. The laminar flow in the capillary channel exerts sufficient shearing forces to break cells and large rigid molecules. We call this "capillary channel" a ***nebulization channel*** and the process of shearing afforded by the formation of this channel, ***nebulization shearing***.

The extent of the shearing force created is proportional to the pressure drop described by the equation of liquid capillary flow. Accordingly, this force is directly proportional to the gas pressure applied and the viscosity of the liquid, and inversely proportional to the size of the droplets. Droplet size can be regulated by gas pressure. The kind of gas used also has an influence on the size of the droplet. Under identical pressure, size of droplets will decrease in following order of gasses used: Argon, Nitrogen, Helium. Thus, the same instrument can be used for very gentle "opening" of the cell as necessary in organelle isolation, or for thorough breakage of the cells, when preparation of a cell extract is desired. Because the size of the formed droplets is very uniform, the applied force is also very uniform resulting in highly efficient cell or molecule breakage.

Breaking cells or shearing molecules in the BioNeb cell disruptor does not generate heat and is achieved in a steady-state manner. Shearing or breakage does not depend on the length of time the shearing forces is applied but only on the magnitude of the force applied i.e., the size of the droplets generated. The operation of this apparatus is simple, rapid, and highly reproducible, as described in the following pages.

What's in this Manual

This manual provides step-by-step procedures for assembly and operation of the **BioNeb® Cell Disruption System** and brief explanations about its features. Technical support data is also included.

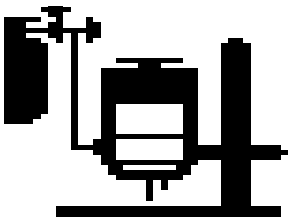
Sections Specific to Cell Disruption

Part I (Description of the Instrument) presents the contents of the BioNeb cell disruptor kit and additional items required to operate the apparatus. **Part II** (Assembly), **Part III** (Calibration), and **Part V** (Detachment and Cleaning) provides step-by-step instructions for each activity. **Part IV** (Operation) presents details about three methods of operation of the BioNeb system.

The appendices contain supplementary information to help you understand the BioNeb system further. **Appendix A** (Feature Diagram) illustrates the function of each component of the unit. **Appendix B** (Worksheet) provides an example of a worksheet that might be convenient while you operate the system. **Appendix C** (Technical Support Data) includes a table presenting the typical distance between the Ball and the Nozzle Cap surface that will produce good misting. **Appendix D** (Tips for Optimal Operation) deals with tips to avoid potential problems you might face during your operation of the BioNeb cell disruptor.

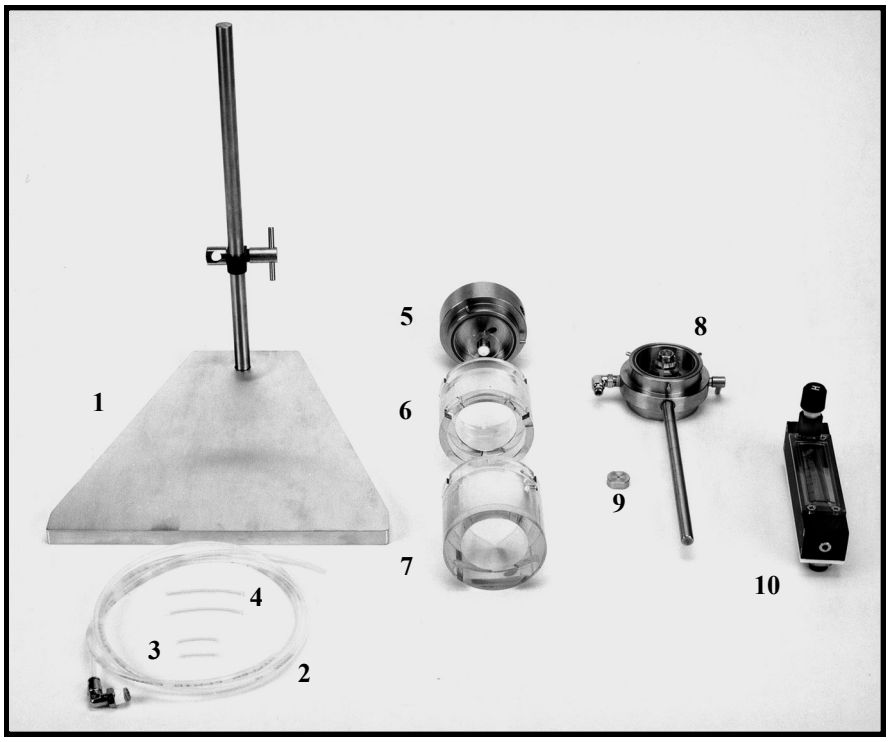
About icon

Throughout the manual you will see an icon of the BioNeb disruptor to help you understand which part of the apparatus is being discussed. The white part of the icon is intended to draw your attention to the part under discussion.



Contents of BioNeb Kit (105A BN3015)

1. Instrument Base
2. Gas Tube
3. Set of Short Inlet and Outlet Tubes
4. Set of Long Inlet and Outlet Tubes
5. Top
6. Cylinder
7. Reservoir
8. Base
9. Nozzle Cap
10. Flowmeter



Additional Items Required Gas tank (Nitrogen, Helium or Argon) with pressure regulator.
Small beakers (several).

Part II. Assembly

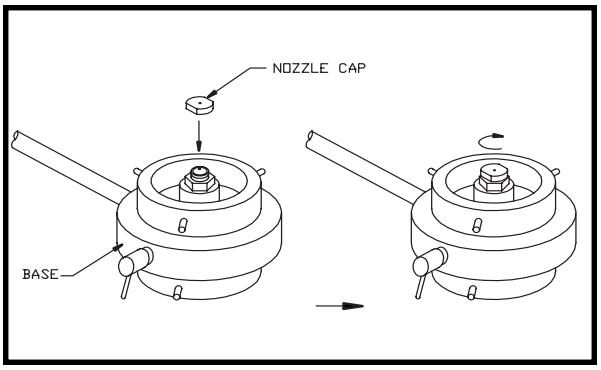
Step 1:

Base Assembly

1.1. Take the base, place the spacer on the nozzle and center it. Screw on the nozzle cap until it stops turning.

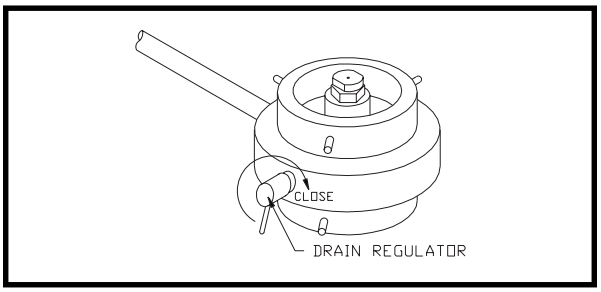
Do it by hand. Never use a wrench because the nozzle cap might freeze on the nozzle!

1 . 1



1.2. Close the drain by turning the drain regulator **clockwise** until it stops.

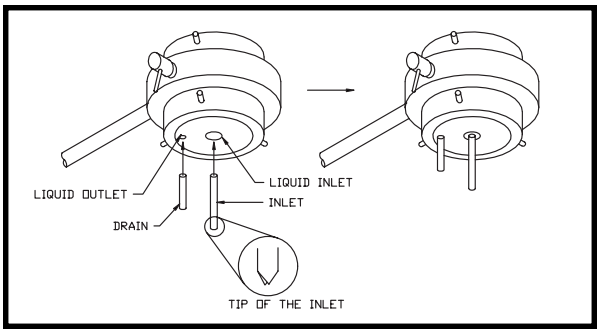
1 . 2



1.3. Insert the long inlet tube into the liquid inlet and the short drain tube into the drain outlet.

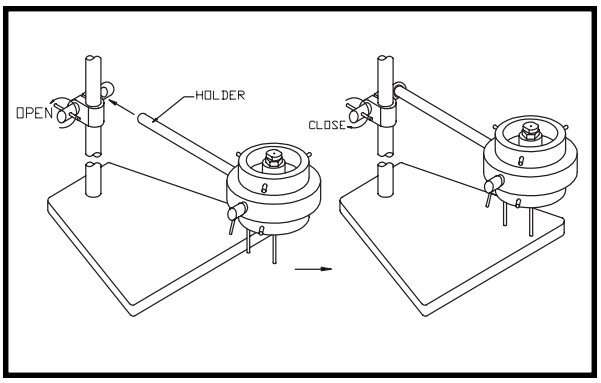
Note: *There are two sets of tubes. The shorter set is for use with reservoir and the longer set is for use with beakers. The tip of the inlet tube is cut as shown in order to facilitate liquid uptake.*

1 . 3



1.4. Insert the holder rod into the opening of the universal instrument positioner.

1 . 4



Step 2:

Cylinder Attachment

2.1. Insert the cylinder into the base so that **each pin rests on the side of the base**.

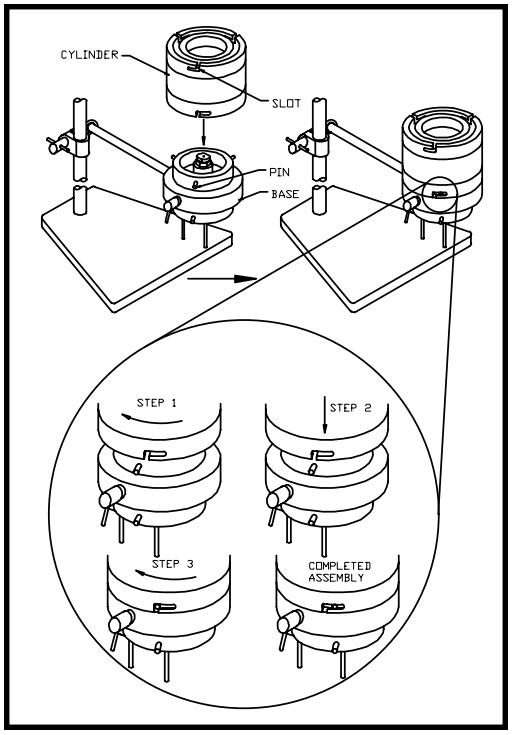
Twist the cylinder until pins are directly above the vertical slots.

Push the cylinder down.

Twist the cylinder until the pins enter the horizontal slots.

Note: The small volume cylinder is attached similarly, but it must be positioned right side up.

2.1



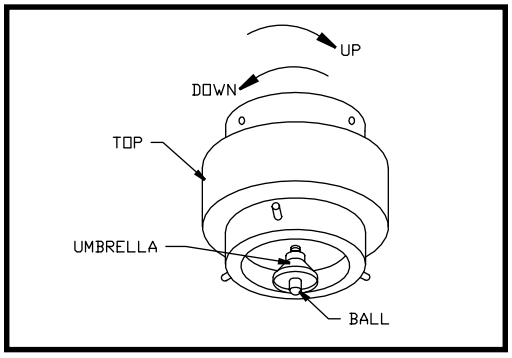
Step 3:

Top Adjustment

3.1. Pick up the top. With the ball securely attached, rotate the umbrella **counterclockwise** until it stops. Now rotate the umbrella **clockwise** for **11** full turns.

Note: *The tightness of rotation of the ball height adjustment disk can be changed. See Appendix A for details.*

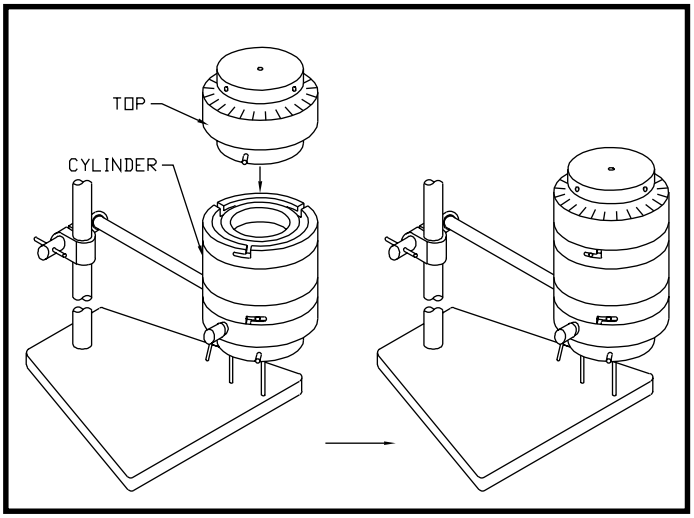
3.1



Step 4:

Top Attachment

4.1



4.1. Insert the top into the cylinder so that **each pin rests on the side of the base**.

Twist the top until pins are directly above the vertical slots.

Push the top down.

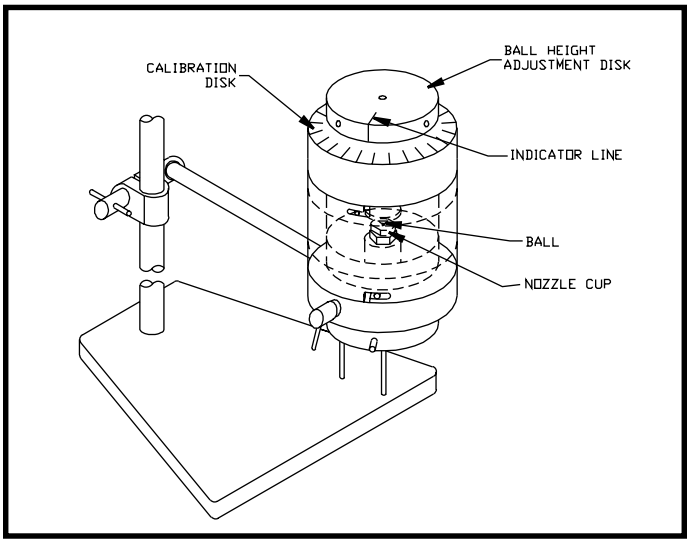
Twist the top until the pins enter the horizontal slots.

The gas outlet should **face away** from the operator. The apparatus should be used in a **chemical or a laminar flow hood.**

Step 5:

Top Adjustment

5.1



Do not force rotation of the nozzle beyond zero position of the ball. You will dent the surface of the nozzle cap.

5.1. Watch the ball while you rotate the ball height adjustment disk **clockwise** slowly until the ball comes in contact with the nozzle cap surface. Stop as soon as you feel resistance.

This is **zero position** for ball height or **BH0**.

***Note**: BH0 will change each time the BioNeb is reassembled.*

Gas Tube Insertion

Push the gas tube into the sleeve at the gas inlet of the base. Pull on the gas tube to make sure that the connection is secure. This completes assembly.

In order to remove the gas line, pull it while pushing in the sleeve of the gas inlet.

The BioNeb is now assembled and ready for calibration.

Part III. Calibration

Step 1:

Gas Tank Setup

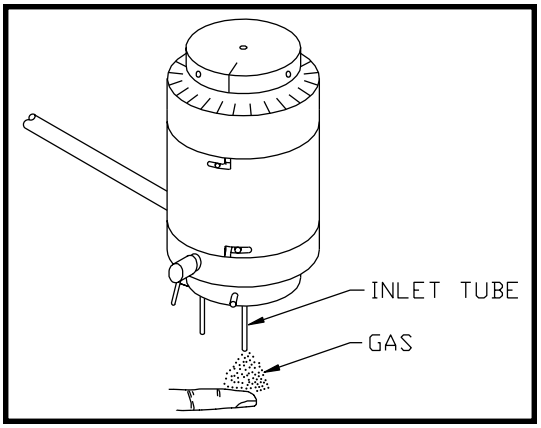
1.1. Open the gas tank valve and adjust the pressure (e.g., 100 psi).

1.1

1.2. Turn the delivery valve 45° from closed position, and note the rapid gas back-flow coming out of the inlet tube. (You can feel it easily with your wet finger.)

Note: *The delivery valve opening can be set differently according to your need.*

1.2

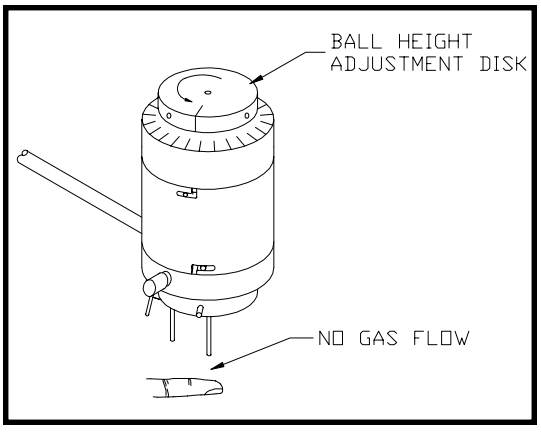


Step 2:

Gas Flow Adjustment

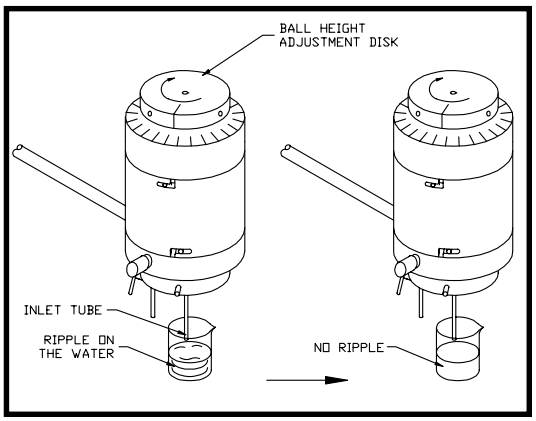
2.1. Rotate the ball height adjustment disk **counterclockwise** until gas back-flow stops.

2.1



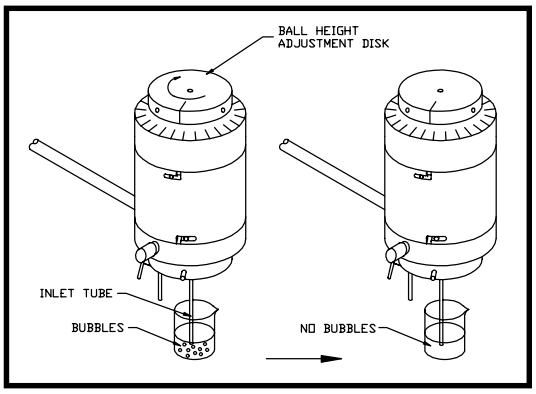
2.2. When the back flow is no longer felt with a wet finger, you can place a small beaker, half filled with water, beneath the liquid inlet tube. Observe the ripple on the water surface caused by the backflow. Rotate the ball height adjustment disk **counterclockwise** until the water surface is no longer disturbed.

2.2



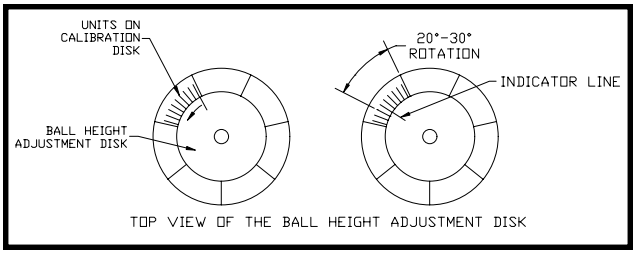
2.3. Immerse the tip of the liquid inlet tube in the water in the beaker and observe slow bubbling. Rotate the disk further until no more bubbles come out. This is **the no bubble position** for ball height or **BHnb**.

2.3



2.4. Continue to rotate the adjustment disk **counterclockwise 4 to 6 units** on the calibration disk (one unit corresponds to 5°). This should give **good misting** and active upward movement of liquid through the inlet tube.

2.4



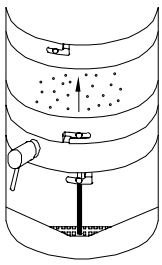
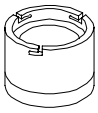
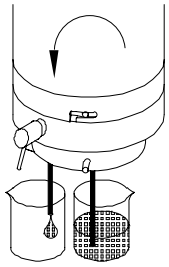
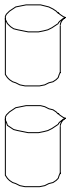
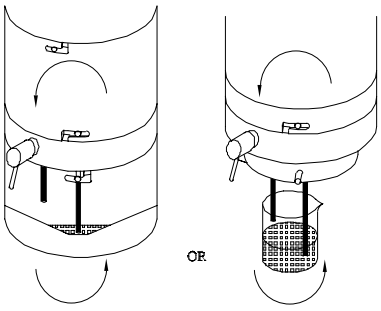
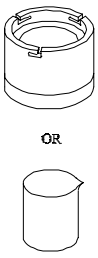
2.5. Turn off the gas tank valve. Turning gas flow on and off with gas tank valve leaves the delivery valve setting constant. This completes the calibration.

2.5

Methods of Operation

The BioNeb can be operated in three different ways:

Method 1: Closed-Drain Single Cycle Mode (Batch Operation)
Method 2: Open-Drain Single Cycle Mode (Batch Operation)
Method 3: Open-Drain Recycling Mode (Continuous Operation)

Mode	Drain Regulator	Liquid Flow	Reservoir
Single Cycle Mode Batch Operation	Closed	Method 1 	
	Open	Method 2 	
Recycling Mode Continuous Operation	Open	Method 3 	

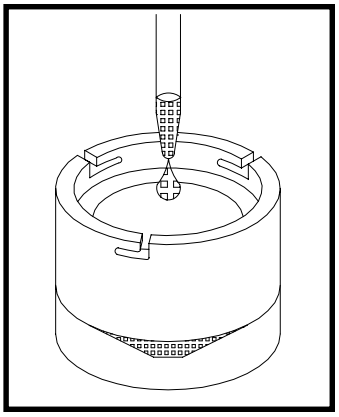
Note: Use the short set of tubes for the BioNeb disruptor reservoir, and the long set of tubes when using beakers.

Method 1:

Closed-Drain Single Cycle Mode

1.1. Place sample solution in the reservoir.

1.1



1.2. Insert the reservoir into the base so that **each pin rests on the side of the base**. Make sure that the short set of tubes is attached.

Twist the reservoir until pins are directly above the vertical slots.

Push the reservoir up.

Twist the reservoir until the pins enter the horizontal slots.

Turn on the gas tank valve. Adjust the ball height adjustment disk, if necessary, to obtain good misting. The initial setting with water may not be optimal for the sample solution. This is **BHexp** position (see Appendix C for further explanation).

After all the sample solution is transferred, turn off the gas tank valve.

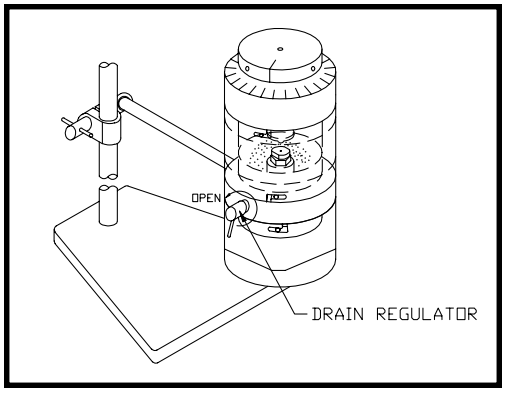
1.2

1.3. Open the drain by turning the drain regulator **counterclockwise** 360° (one full turn).

Note: *For multi-cycle operation, repeat steps 1.3 to 1.5.*

Detach the reservoir and collect the sample.

1.3

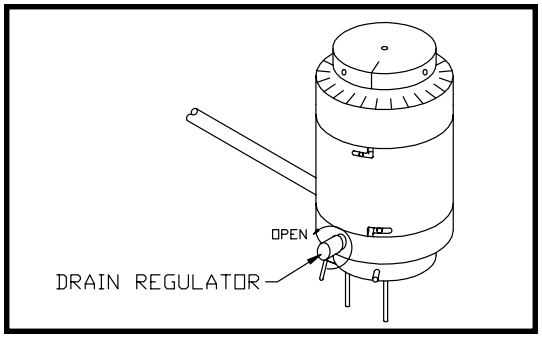


Method 2:

Open-Drain Single Cycle Mode

2.1. Open the drain by turning the drain regulator **counterclockwise** 360° (one full turn).

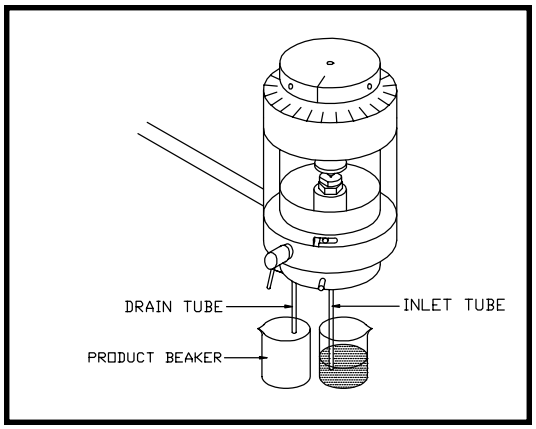
2.1



2.2. The inlet tube is inserted into the sample solution while the drain tube is inserted into the beaker. Make sure that the long set of tubes is attached.

Turn on the gas tank valve. Adjust the ball height adjustment disk, if necessary, to obtain good misting. The initial setting with water may not be optimal for the sample solution. This is **BHexp** position (see Appendix C for further explanation).

2.2

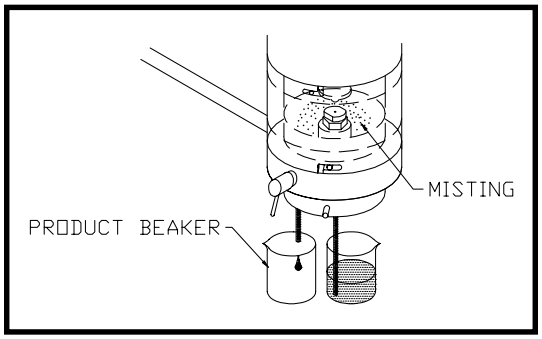


2.3. With **good misting**, sample solution should be transferred to the product beaker.

When all the sample solution is transferred to the product beaker, turn off the gas tank valve.

Note: *For multiple-cycle operation, repeat the steps 2.2 to 2.5.*

2.3

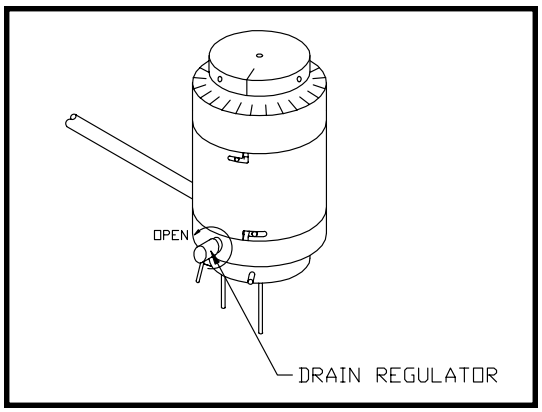


Method 3:

Open-Drain Recycling Mode

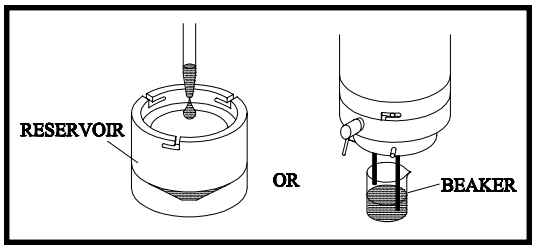
3.1. Open the drain by turning the drain regulator **counterclockwise** 360° . Use the short set of tubes for the reservoir and the long set of tubes for the beaker.

3.1



3.2. Place the sample solution into the reservoir (or beaker).

3.2



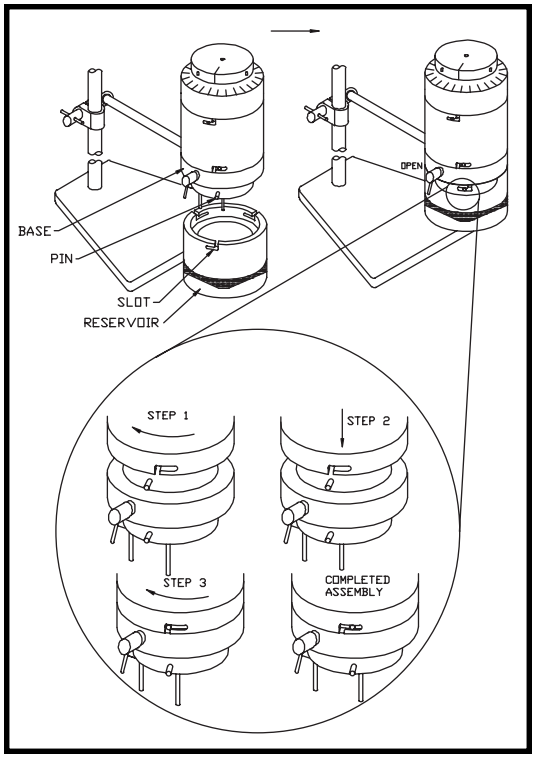
3.3. Insert the reservoir into the base so that **each pin rests on the side of the base**. Make sure that the short set of tubes is attached.

Twist the reservoir until pins are directly above the vertical slots.

Push the reservoir up.

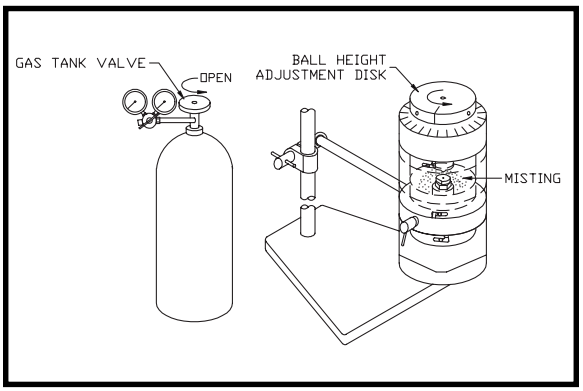
Twist the reservoir until the pins enter the horizontal slots.

3.3



3.4. Turn on the gas tank valve. Adjust the ball height adjustment disk, if necessary, to obtain good misting. The initial setting with water may not be optimal for the sample solution. This is **BHexp** position (see Appendix C for further explanation).

3.4



Nebulize for a given period (e.g., 5 minutes) and then turn off the gas tank valve.

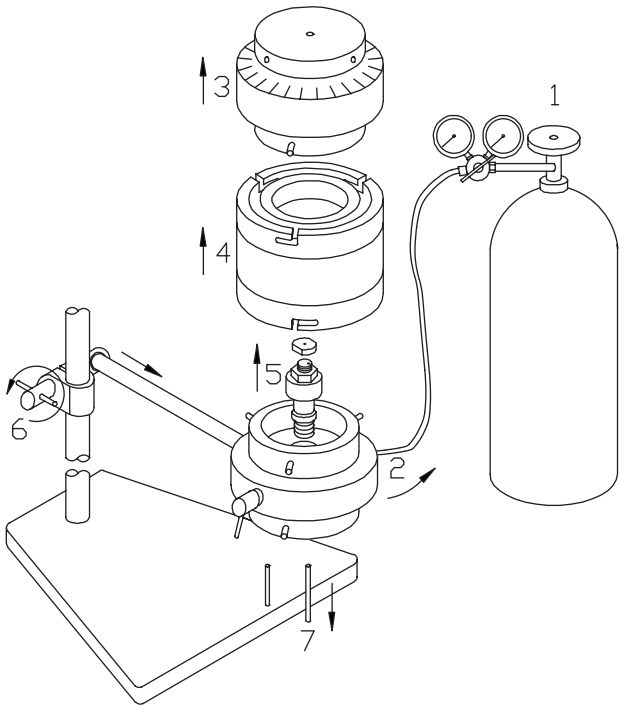
Twist the reservoir until pins are directly above the vertical slots.

Push the reservoir down.

Detach the reservoir and collect the sample.

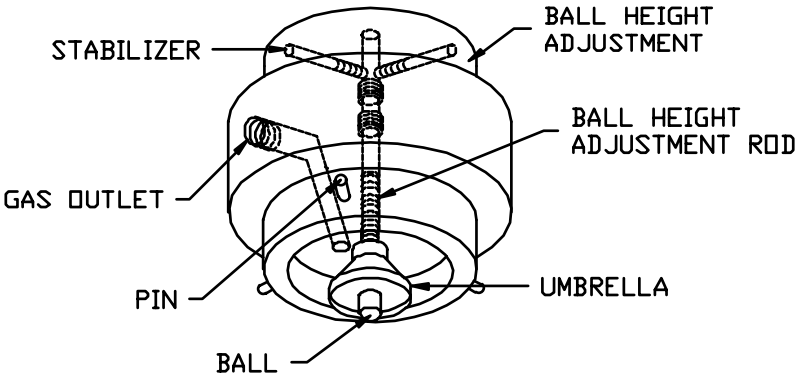
Part V. Detachment and Cleaning

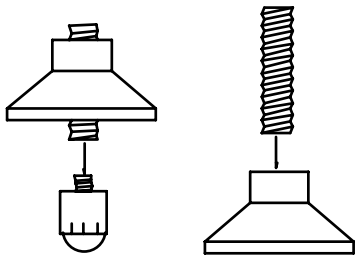
1. Make sure that both the main gas valve and the delivery valve are closed.
2. Remove the gas line by pulling it while pushing into the sleeve of the gas inlet.
3. Detach the top, rinse and dry. (Ethanol can be used but not acetone!)
4. Detach the cylinder, rinse and dry.
5. Unscrew the nozzle cap from the base, remove the spacer, rinse and dry.
6. Rotate the knob of the universal instrument positioner **counterclockwise** and detach the base from the positioner, rinse and dry. Make sure that both liquid and gas passages are clear.
7. Detach liquid inlet tube and drain tube, rinse and dry.



It is recommended that all components of the BioNeb be stored clean and dry. Do not leave the instrument not cleaned after usage.

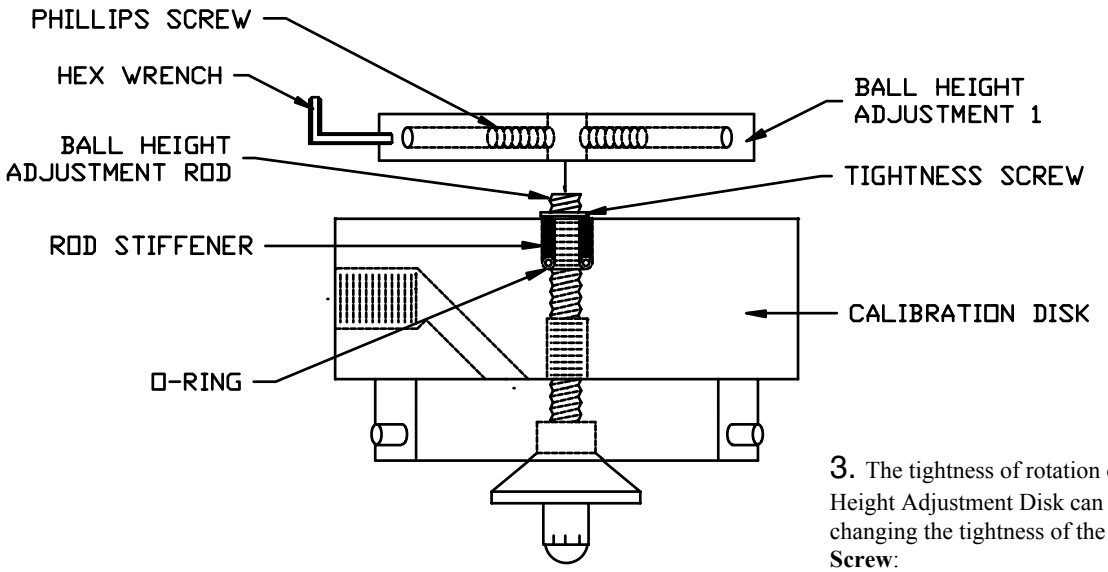
BioNeb Cell Disruptor - Top





1. The Ball is detached from the Ball Height Adjustor Rod.

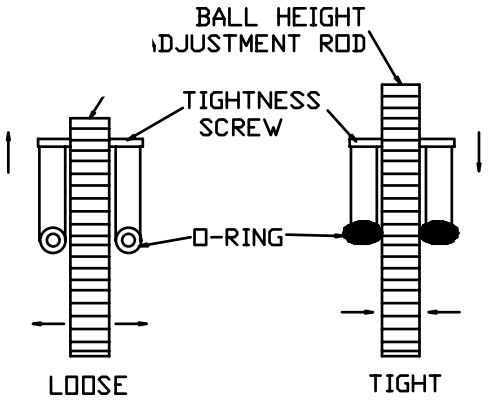
2. The Umbrella is removed from the Ball Height Adjustor Rod.



3. The tightness of rotation of the Ball Height Adjustment Disk can be adjusted by changing the tightness of the **Tightness Screw**:

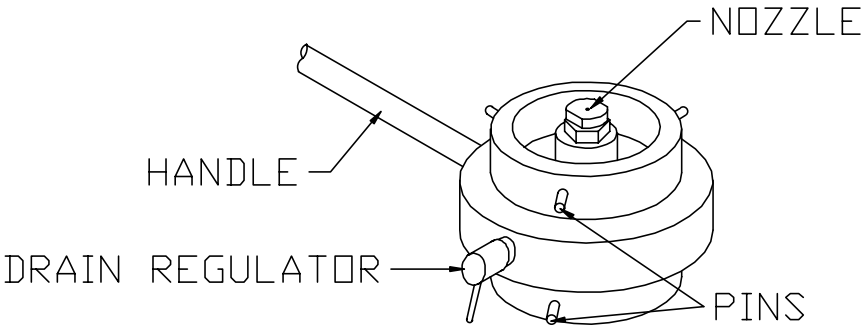
(a) Turn three Phillips Screws by using a hexa-wrench. The Ball Height Adjustment Disk is detached from the Calibration Disk.

(b) Turn the **Tightness Screw** on the Calibration Disk to apply pressure on the O-ring. Alteration of the O-ring shape will change the tightness of rotation.

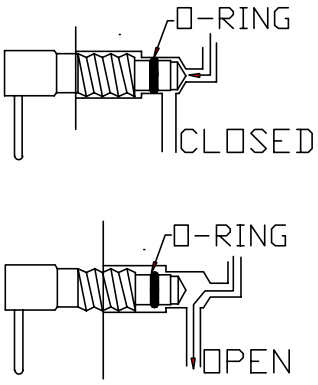


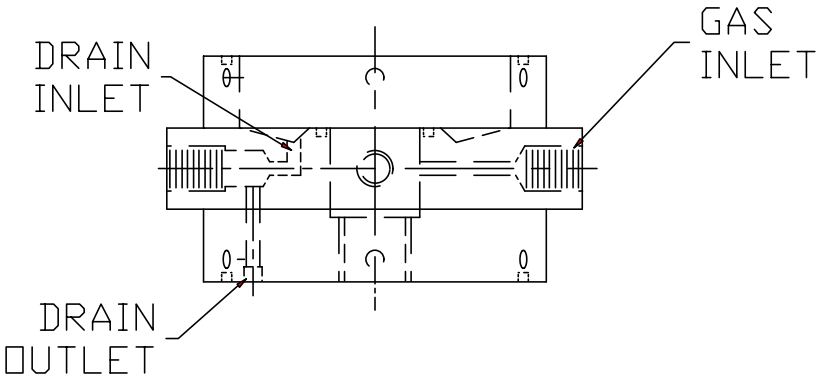
BioNeb Cylinder (large volume)

BioNeb Base

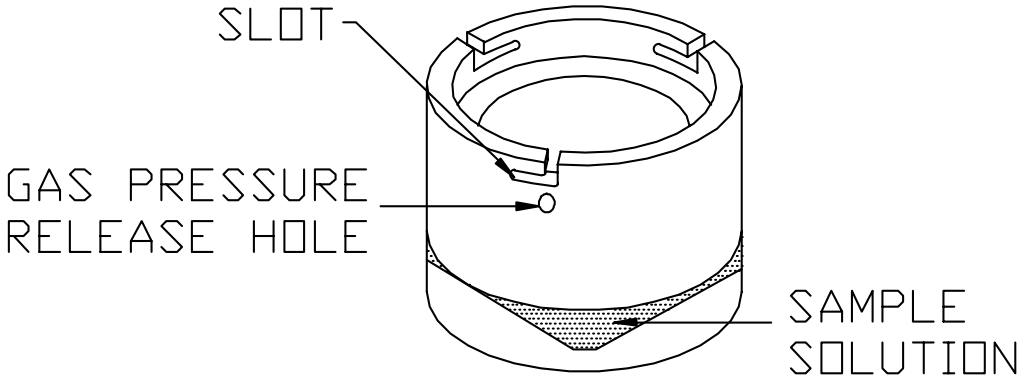


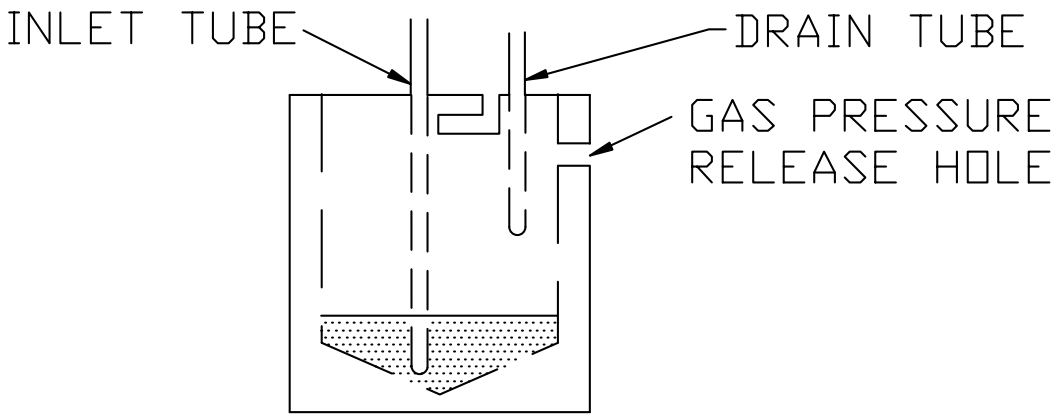
4. Drain Regulator Operation:





BioNeb Reservoir





Appendix B Worksheet

Date:

Objective:

Organism:

Protocol:

Exp. No.	No. of Cycles	Type of Gas	PSI	Gas Delivery Valve Turn	BH_0	BH_{exp}	DBH $(BH_{exp} - BH_0)$	Experiment Notes
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Initial gas tank pressure: Final gas tank pressure:

The difference between **BH_{exp}** and **BH_0** (**BH_{exp} - BH_0**) is **DBH** which is the indicator of distance between the Ball and the Nozzle Cap surface. This number should be relatively constant for a given experimental setting; i.e., under the same gas pressure.

For your convenience, the top of the Calibration Disk is divided into 72 engraved lines, with tick marks at every 10 lines, except between line 70 and line 72/0. It is recommended that each tick line be numerically marked (0, 10, 20, ... 70), for easy identification.

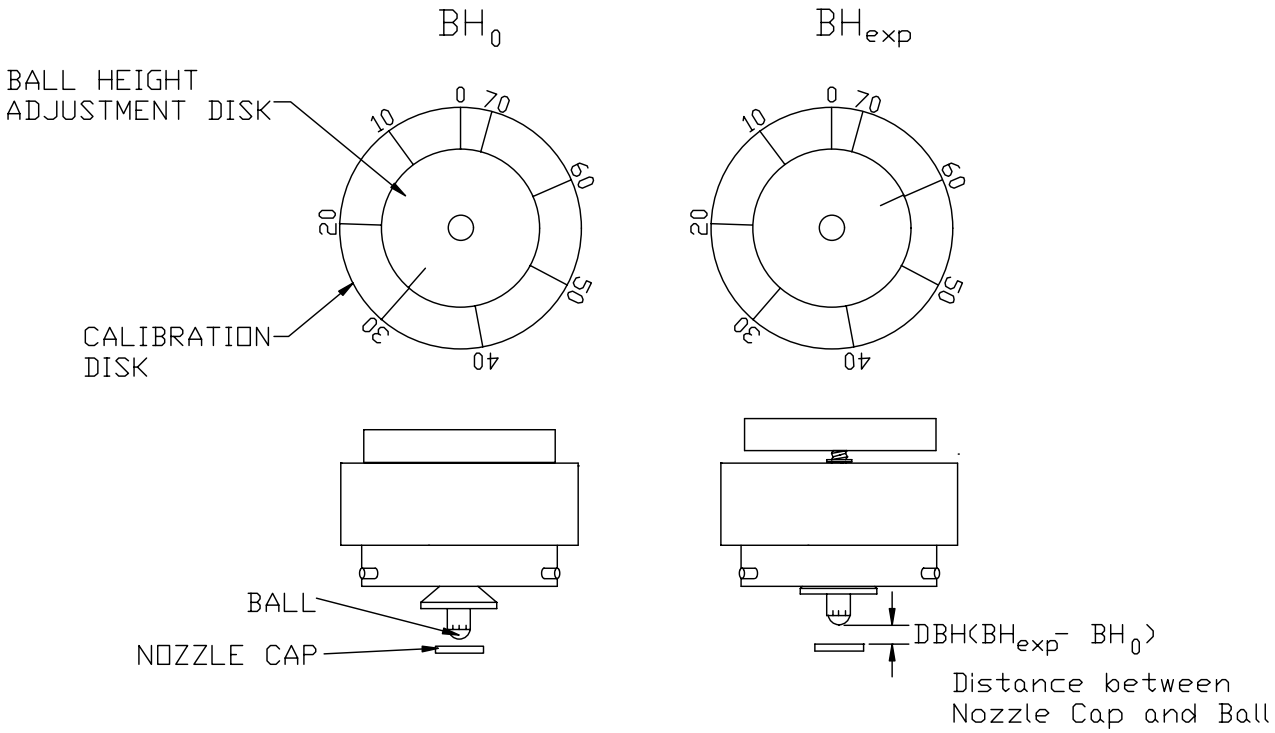


Table. Typical Distance Between the Ball and the Nozzle Cap Surface

Solution Nebulized	Type of Gas	Gas Pressure (psi)	Gas Delivery Valve Turn	BH_0*	BH_{nb}	BH_{exp}	ĐBH (BH_{exp} - BH_0)	(BH_{exp} - BH_{nb})
Yeast	Helium	200 psi	1/8 turn	33	61	67	34	6
"	"	150	"	46	60	66	20	6
"	"	100	"	43	58	64	21	6
"	"	50	"	50	58	64	14	6
Yeast	Nitrogen	200 psi	1/8 turn	27	14	20	65	6
"	"	150	"	26	65	71	45	6
"	"	100	"	28	60	66	38	6
"	"	50	"	25	53	59	34	6
Yeast	Argon	200 psi	1/8 turn	21	66	0	51	6
"	"	150	"	25	65	71	46	6
"	"	100	"	30	60	66	36	6
		50	"	28	55	61	33	6

* BH_0 will change each time when the BioNeb is reassembled.

Alternative Regulation of Gas Flow by a Flow Meter

Theoretically, the efficiency of nebulization should be proportional to the flow rate of gas exiting from the nozzle. In practice, the efficiency of cell breakage with helium, nitrogen and argon have shown a linear relationship with gas flow rate within certain ranges.

Furthermore, one can control gas flow rate more precisely with a flow meter. With Cole Parmer flow meter #054-17ST, we carried out 3 cycles of nebulization of yeast cell suspension under 200 psi and found the following ranges of gas flow rate that give proportional cell breakage.

Helium	(10,000 ml/min. to 20,000 ml/min.)
Nitrogen	(2,500 ml/min. to 8,500 ml/min.)
Argon	(2,500 ml/min. to 6,500 ml/min.)

Potential Problem: Instead of good smooth misting, spattering occurs.

Solution: Clean the nozzle opening, as well as the nozzle cap opening regularly.

Potential Problem: Variable gas pressure might decrease the efficiency of cell breakage.

Solution: Check the gas pressure remaining in the tank.

Potential Problem: Loose coupling of pins and slots of the Base and the Cylinder might cause a liquid leak.

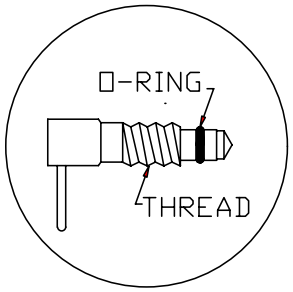
Solution: Check the tightness of the coupling between the base and the cylinder. If necessary replace O-rings with new ones.

Potential Problem: Insufficient seal might develop a gas leak at the gas inlet during operation.

Solution: Reseal the gas inlet with fresh Teflon® resin thread seal tape.

Potential Problem: The drain regulator might become hard to turn.

Solution: Apply stopcock grease to the thread of the Drain Regulator regularly. Make sure that stopcock grease does not reach beyond the O-ring.



Drain Regulator

WARNING:

1. **Do not open gas tank valve before the gas tube is inserted into the BioNeb system.**

2. **Operate the BioNeb disruptor in a chemical hood to avoid contamination of laboratory space by nebulized materials.**