Tube Piercer

Installation and Operation Guide

TELEDYNE ISCO
A Teledyne Technologies Company

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Tube Piercer Installation and Operation

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Section 1 Introduction

1.1 Density Gradient Centrifugation

The principle of density gradient centrifugation has long been regarded as a powerful tool in biological science. It allows the separation of large molecules or small particles based entirely upon their sedimentation characteristics. It also allows the precise measurement of sedimentation rates in strong centrifugal fields making it possible to calculate many useful parameters of the material being studied.

Equilibrium density centrifugation separation is accomplished by constructing a gradient of material that will increase in density from the top to the bottom of the centrifuge tube. For this application, the gradient is made so that the density extremes will encompass the densities of all the particles being studied in the sample. By applying the sample to the top of the gradient and centrifuging, particles will migrate to that point in the gradient which is equal to their own density. At this point, they will stop and be separated from other dissimilar particles. Many cellular organelles and other particles have a characteristic density, which allows equilibrium separations in gradients to be achieved. This method can yield fractions of extremely high purity.

Rate density gradient centrifugation is probably the most commonly used technique and has wide practical application. With this technique, the sample is layered on the top of the density gradient column and centrifuged for a set time period. The sedimenting components do not reach zones of equal density in the tube before the centrifugation is stopped, but rather the separation of the different particulate species occurs in zones as a result of different sedimentation rates.

The following references provide useful information on the preparation of density gradients for centrifugation: Brakke, **Archives of Biochemistry and Biophysics,** 107, 388-403 (1964); and Brakke, **Analytical Biochemistry,** 5, 271-283 (1963).

1.2 Chemical Compatibilities

Materials in contact with the liquid stream are: glass, PTFE, vinyl, stainless steel, and Delrin.

⚠ CAUTION

Solutions which are not compatible with all of these materials should not be used.

Styrene-butadiene rubber is used in the seals at the top and bottom of the tube, but are not in direct contact with the liquid stream; a slight incompatibility can be tolerated.

The tube piercing system is constructed of Delrin and stainless steel and is quite resistant to water and mild detergent solutions which may be used to clean it.

1.3 Tube Piercer

The Tube Piercer is a useful accessory for density gradient centrifugation work. It may be used in conjunction with a well-regulated pump to inject a dense solution into the bottom of the tube containing the centrifuged density gradient, raising the contents of the tube upward through the universal top holder or flow cell of an absorbance detector (see Figure 1-2).

Figure 1-1 Tube piercer with centrifuge tube and flow cell

The flow cell, or universal top holder, is mounted directly above the centrifuge tube to prevent inversion of the gradient and has an inlet cone, which minimizes mixing. Additionally, the flow cell has large flow passages which eliminate mixing due to the laminar or turbulent flow that takes place in smaller passages, even when stabilized by a density gradient.

Either the flow cell or top holder will accommodate most sizes of centrifuge tubes through the use of several interchangeable styrene-butadiene rubber collars, which also provide a liquid seal between the flow cell and the centrifuge tube. Different tube lengths are accommodated by a vertical adjustment of the piercing mechanism.

The contents of the tube may be fractionated in one of two ways. The tube is pierced from the bottom by the spring-loaded piercing mechanism, as shown in Figure 1-2.

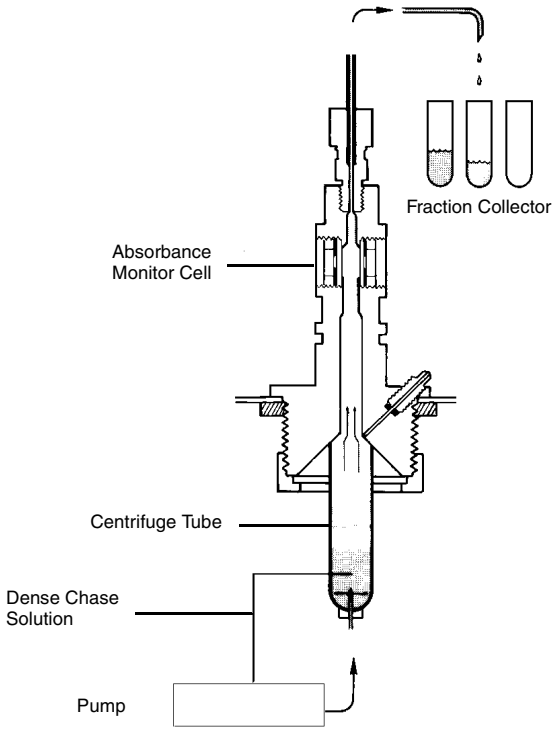


Figure 1-2 Basic fractionation procedure

1.4 Tube Piercing System

The tube piercing system, shown below in Figure 1-3, is composed of three parts: the flow cell, the bottom holder, and the tube piercing apparatus. The flow cell holds the centrifuge tube in place at the top through the use of a rubber collar.

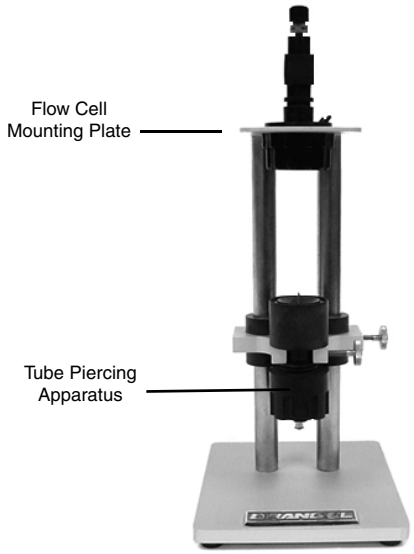


Figure 1-3 Tube piercing system

The bottom holder is made of Delrin and mounts on top of the piercing apparatus. It is round and has a concave, conical inside surface that forms a base to support the bottom of the centrifuge tube. A trough runs around the periphery of the bottom holder to catch any excess liquid spillage. A small vertical hole is located in the center through which the needle passes, and a larger bottomed hole that forms the seat for the rubber gasket seal (septum).

The tube piercing assembly is shown in Figure 1-4. It is adjustable vertically on the two rods on which it rides, by loosening the rear thumbscrew. It can be removed by loosening the front thumbscrew.

⚠ CAUTION

Tubes made of materials such as polycarbonate and glass cannot be pierced.

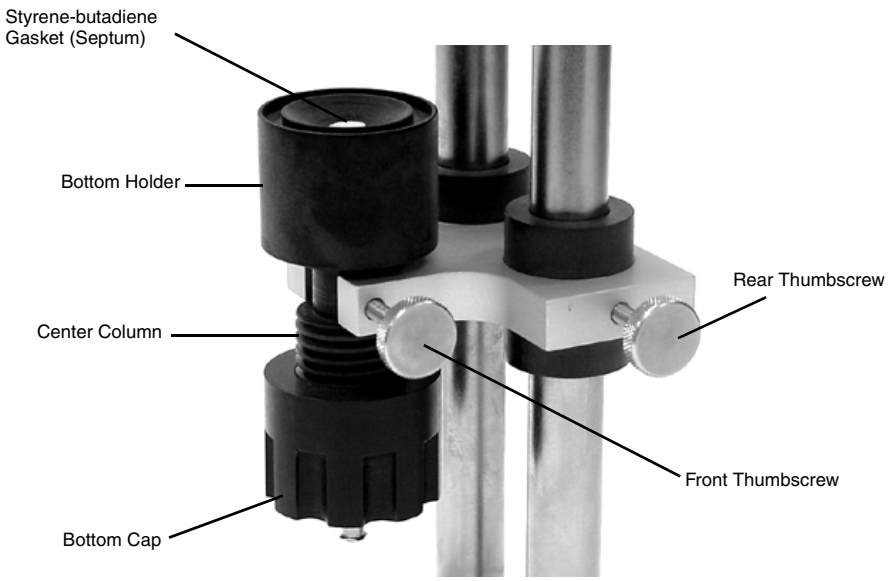


Figure 1-4 Piercing assembly

Mechanically, the apparatus is simple. It is composed of three parts: a threaded bottom cap which mounts the piercing needle and a center column which mounts the bottom holder on its top and is threaded at its lower end to accept the threaded cap. When the threaded cap is twisted on the center column, the needle advances vertically, puncturing the centrifuge tube.

The bottom holder is spring loaded by a spring in a slot on the top of the center column. The spring loading assures a tight seal between the bottom holder and the centrifuge tube.

The puncturing needle is mounted to a stainless steel disk that is free to rotate within the threaded cap. A vertical rod, which is mounted rigidly to the disk, extends into a hole in the center column restraining the needle from turning when the threaded cap is turned. This prevents the needle from forming an enlarged hole in the centrifuge tube and also prevents twisting of the connect tubing from the syringe. A male Luer-Lok fitting in the bottom of the disk forms the entrance to the liquid flow path leading to the needle.

Tube Piercer Installation Guide

Section 2 Operating Procedures

The following is a description of the procedures for the actual scanning and fractionating process. It is strongly recommended that this section be carefully read before scanning a tube.

2.1 Adjustments for Tube Sizes

It is necessary to make adjustments in two areas of the tube piercer to compensate for different sizes of centrifuge tubes: The flow cell or universal top holder, and the piercing mechanism. These two areas are discussed in the following sections.

Table 2-1 Table of Centrifuge Tube Sizes

Tube Size	Mfg. Rotor Designation	Collar and Ring Designation
7/16 x 1-15/16	Beckman	A
7/16 x 2-3/8	Beckman SW 56	A
10.9 x 54.7 mm	International SB 405	A
1/2 x 2	Beckman SW 39, 50, 65, 50.1	B
1/2 x 2-1/2	Beckman Type 40.2 and 40.3	B
12.7 x 50.8 mm	International 2865	B
12.7 x 98.4 mm	International	B
9/16 x 3-1/2	Beckman SW 41	C
9/16 x 3-3/4	Beckman SW 40	C
14.5 x 96 mm	International SB 283 and 206	C
14.5 x 102 mm	International	C
5/8 x 2-1/2	Beckman 50	D
5/8 x 3	Beckman Type 40 TI-50	D
5/8 x 4	Beckman SW 25.3	D
16.1 x 76.2 mm	International 495	D
23 x 70 mm	MSE 23 ml	F Collar / E Ring
1 x 3	Beckman SW 25.1	E
1 x 3-1/2	Beckman SW 27 and Type 30	E
25.4 x 88.9 mm	International SB-110	E
1-1/4 x 3-1/2	Beckman SW 25.2	Uncoded
1/2x 2	Beckman Quick-Seal® 342412	G Collar / F Ring
5/8 x 3	Beckman Quick-Seal® 342413	G
1 x 3-1/2	Beckman Quick-Seal® 342414	H

⚠ CAUTION

Tubes made of materials such as polycarbonate and glass cannot be pierced.

2.2 Flow Cell

The flow cell (Figure 2-1) is designed in such a way that the basic flow cell, through the use of interchangeable rubber collars, will accommodate a variety of centrifuge tubes ranging in size from 7/16 inch (11.11 mm) to 1¼ inch (31.75 mm) diameter. The flow cell can be ordered with any one of three optical path lengths: 2, 5, or 10 mm. Slit apertures are supplied to limit the illuminated volumes. A 5 mm path length provides optimum resolution for most applications. The 2 mm path length is useful if a highly absorbing material is present, and the 10 mm path length provides maximum sensitivity.

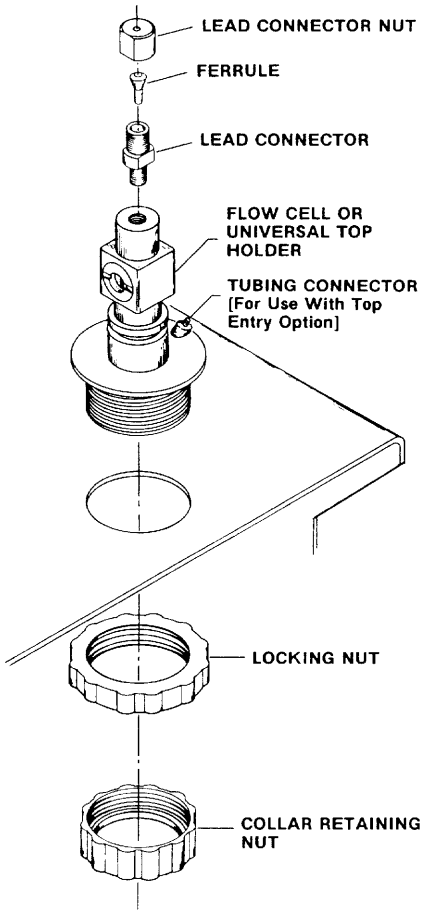


Figure 2-1 Flow cell assembly

The standard apertures, which are installed in the flow cell, have a 1.0 mm high horizontal slit. Included in the flow cell accessory package are two apertures that have a slit height of 2.8 mm. These apertures may be installed in the flow cell to increase the illuminated volume.

The larger (2.8 mm) aperture is installed in the flow cell in the following manner (Figure 2-2). First, remove both window nuts using the wrench included with the flow cell. Then, remove the

smaller apertures and install the larger apertures in their place. Reinstall the window nuts and tighten. The light aperture slits must be in the horizontal plane as shown in Figure 2-2.

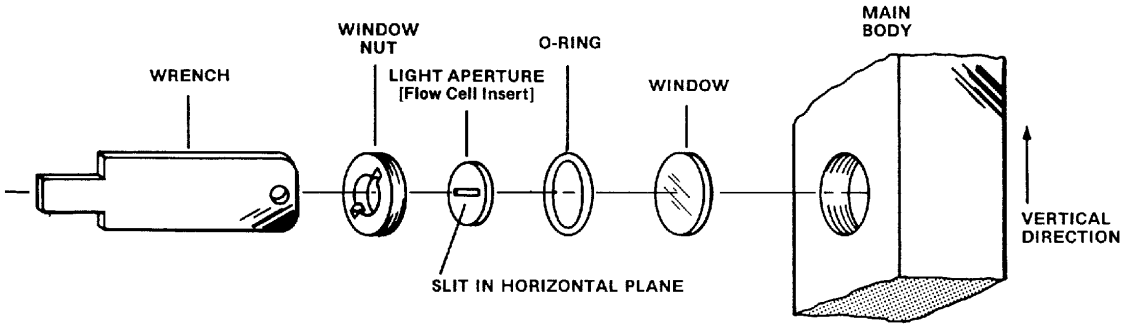


Figure 2-2 Flow cell aperture installation

☑ Note

The illuminated volumes are specified in Table 2-2, as well as the light aperture insert heights and path lengths.

Table 2-2 Flow Cell Illuminated Volumes

Light Aperture (mm height)	Path Length (mm)	Illuminated Volume (µl)
1.0	2 5 10	6 14 28
2.8	2 5 10	15 39 78

Each flow cell will accommodate a wide variety of centrifuge tubes through the use of the interchangeable collars (Section 2.4). These collars are made of styrene-butadiene rubber and a set of eight is included with each flow cell.

The eight collars are designed to accommodate the most commonly used centrifuge tube diameters as shown in Table 2-1. All of the collars are cone shaped, with the exception of the ring shaped collars for 1¼ inch diameter tubes. Each collar, with the exception of the two with 1¼ inch diameters, has a stainless steel ring coded with a similar letter.

2.3 Flow Cell/ Universal Top Holder

To install the flow cell or universal top holder in the instrument, first remove the collar retaining nut (Figure 2-1). Then remove the locking nut. Place the flow cell through the hole in the flow cell mounting plate. Reinstall the locking nut and tighten. The collar retaining nut may now be placed on the flow cell.

2.4 Selection and Installation of Collar

To install a collar in the flow cell, remove the retaining nut from the flow cell (Figure 2-3). Select the proper size collar and ring (if used) from Table 2-1 and install them in the groove on the inside of the retaining nut as shown in Figure 2-3.

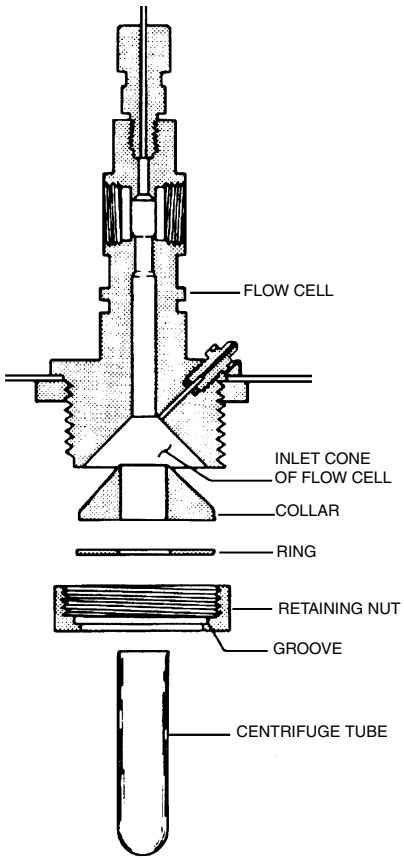


Figure 2-3 Collar installation

The collars and bottom holder septa are shipped in a septum and collar set (Part #68-0643-266), shown below. This is done to avoid the possible misplacement of any of these relatively small parts.

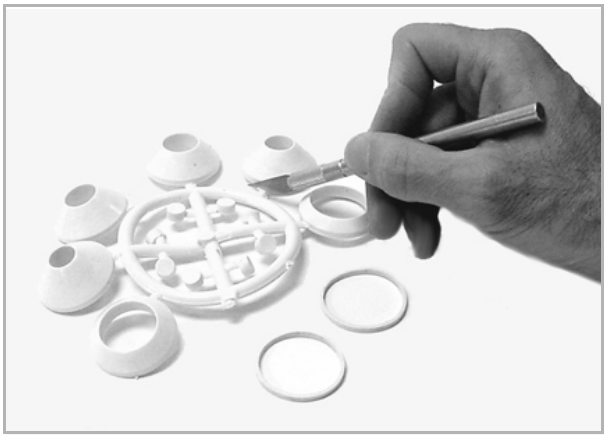


Figure 2-4 Septum and collar set

Before any of the individual parts can be used, they must be separated from the set. This is done by cutting the small tab connecting the part to the rest of the set with a small, sharp knife, as shown in Figure 2-4. After the part has been separated from the set, the tab area should be trimmed to give the part a smooth contour.

Finally, loosely screw the retaining nut and collar assembly back into the flow cell, as shown in Figure 2-3.

> ☑ ***Note***
>
> For 1¼ inch diameter tubes, the proper procedure is to remove the retaining ring from the flow cell. Place the collar around the top of the tube, and slide the retaining ring onto the tube from the bottom. Carefully secure the retaining ring, collar, and tube to the flow cell.

Centrifuge tubes not found in Table 2-1 may possibly be accommodated by one of the eight collars. Simply select the best fitting collar which will provide a seal.

In actual use, the retaining nut is loosened slightly and the centrifuge tube is pushed up into the collar until it is in contact with the inlet cone of the flow cell. The retaining nut is then tightened, squeezing the collar around the outside of the centrifuge tube and providing a liquid seal. When the fractionation process is finished, the chase solution is first lowered below the top of the centrifuge tube by reversing the flow of the pump, and then the retaining nut is loosened for removal of the tube.

2.5 Filling the Chase Liquid System

The chase liquid system must be purged of air and filled with chase solution before the actual fractionating process can proceed. The chase liquid must be more dense than the solution at the bottom of the gradient in the centrifuge tube.

Fluorinert FC40 electronic liquid (part #68-0647-021) is a very satisfactory chase liquid for all common gradient materials. Sucrose solutions are widely used as a chase liquid; however, sucrose solutions more concentrated than 1.8M (620g per liter) are too viscous to be forced through the small orifices of the system and should not be used.

Sucrose solutions may be used to chase sucrose, glycerine, Ficoll, or dextran gradients, but cannot be successfully used alone to chase dense solutions of salts such as NaBr or CsCl, nor can the salts be used to chase sucrose. Convection and disruption of the bottom of the gradient column occur if sugar solutions are chased with salt solutions or vice versa. This convection is apparently a result of the widely different diffusion rates of salt and sucrose and the resulting loss of salt from the salt solution next to the sucrose solution. The use of Fluorinert is highly recommended in all cases.

Any type of pump may be used with the tube piercer to inject the dense chase solution into the bottom of the centrifuge tube. A low pulsation pump such as the Isco Tris is recommended for this.

The chase solution connection on the bottom of the tube piercer is a female Luer-Lok fitting. A length of vinyl tubing with a male Luer-Lok on one end is supplied with the tube piercer. It can be used to connect the tube piercer to the pump used.

Remove the bottom holder from the piercing mechanism to expose the needle tip and outlet holes, and the needle filled with chase liquid. This is done by slowly pumping the chase liquid into the needle until chase liquid comes out of the needle outlets. Wipe up any spilled liquid. Care should be taken to always purge the liquid system of air bubbles, or the rising bubbles may disturb the zones within the gradient.

2.6 Installing and Piercing the Tube

After the pump has been filled and the connecting tube and needle purged, the centrifuge tube may be installed in the flow cell.

The rubber disk gasket in the bottom holder prevents leakage after the needle puncture. It can be covered with either a silicone grease or white petroleum jelly before the tube is punctured, to aid in sealing. The gasket will eventually wear out from repeated punctures and have to be replaced with one of the spares included with the instrument. It should be visually checked before each run.

Select the proper rubber collar and corresponding ring from Table 2-1 and install in the flow cell as shown below.

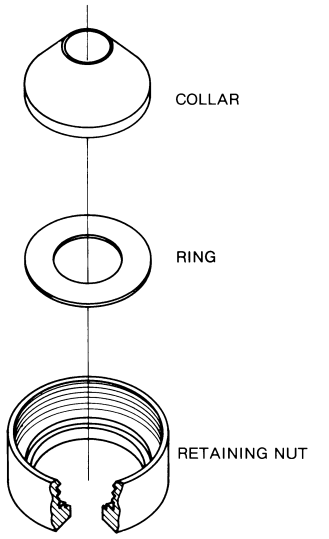


Figure 2-5 Collar and ring installation

Loosen the retaining nut on the bottom of the flow cell to relieve any compression on the rubber collar. Then, carefully push the centrifuge tube up into the hole in the collar until it is felt to make contact with the bottom of the flow cell.

Finally, holding the tube with one hand, tighten the retaining nut with the other (Figure 2-6). The nut should only be snugged up; over-tightening may cause the tube wall to ripple and leak. Check to insure that the centrifuge of the tube is vertical; if it is not, push it into a vertical position.

Figure 2-6 Installation of the centrifuge tube

Turn the threaded bottom cap on the piercing apparatus to the left until it is almost to the bottom of the threads on the center column. Then, loosen the rear thumbscrew of the piercing apparatus and raise the piercer until the bottom holder gasket makes firm contact with the bottom of the centrifuge tube (Figure 2-7).

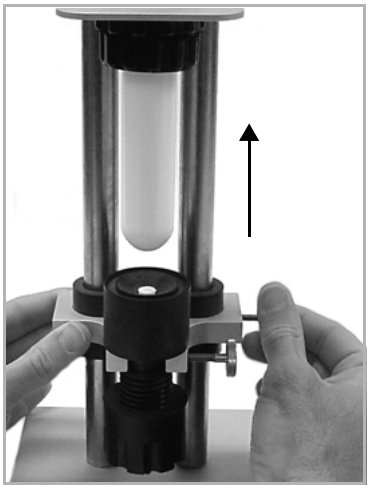


Figure 2-7 Adjusting the height of the tube piercer

Continue raising it until the bottom holder is lowered approximately 5 millimeters ($^3/_{16}$ of an inch) on its spring, assuring a tight seal between the gasket and the tube. Lock the piercing apparatus in place by retightening the rear thumbscrew. The centrifuge tube is pierced by turning the threaded cap to the right (Figure 2-8), raising the needle.

Figure 2-8 Piercing the centrifuge tube

Observe the progress of the needle into the tube and stop turning the cap when the holes (not the tip) in the needle are just above the bottom of the centrifuge tube. If the threaded cap cannot be turned any more and yet the holes in the needle are not inside the tube, loosen the rear thumbscrew, and raise the entire piercing until the needle holes are in their proper positions. The gradient is now ready to be scanned and fractionated.

2.7 Scanning the Gradient

After the centrifuge tube has been pierced, the scanning process can be started. Prior to this, an Isco UA-6 absorbance detector optical unit should be attached to the flow cell. Refer to the UA-6 instruction manual for operating instructions.

☑ *Note*

Care should be taken not to trap air bubbles in the needle as they will disrupt the gradient if they pass into the tube. To avoid retaining air bubbles, the needle should be purged with sucrose or chase solution and the plastic supply tube should not be manipulated while the centrifuge tube is being placed in the apparatus or during the piercing operation.

The initial flow of the dense chase solution should be at a slow flow rate to avoid disturbing any pellet in the tube and to maintain a sharp interface between the tube contents and the chase solution.

Suggested scanning speeds are 3.00 to 6.00 ml/minute for large tubes (e.g., $1\frac{1}{4}$ x $3\frac{1}{2}$ inch tubes), 1.50 to 3.00 ml/minute for medium size tubes (e.g., 1 x 3 inch tubes), and 0.375to 0.750 ml/minute for small tubes (e.g., $\frac{1}{2}$ x 2 inch tubes).

Faster flow rates than these will lead to complications for the following reasons:

1. If a constant speed recorder is used, it may give a compressed scanning curve for high flow rates, resulting in poorer apparent resolution of peaks.
2. If a fast flow rate is used with very viscous solutions, pressure may build up in the system and leaks may occur.
3. Turbulence or laminar flow may occur at higher flow rates, causing a decrease in resolution observed at the absorbance monitor.
4. The recorder pen moves slowly and since the rate of change in optical density will increase with faster speeds, recording inaccuracies and poorer apparent resolution may result.

2.8 Fraction Collecting

The fraction collector preparations should be set up prior to the beginning of the scanning process. However, the fraction collector need not be turned on until liquid first appears from the discharge tube.

The output of the flow cell may be delivered to the fraction collector through either $\frac{1}{8}$ inch (3.18 mm) outside diameter, 0.022 inch (0.56 mm) inside diameter PTFE tubing, or 0.046 inch (1.17 mm) outside diameter by 0.022 inch (0.56 mm) inside diameter PTFE tubing. Either tubing, with their appropriate ferrule, will work equally well with the flow cell. The tubing used for any particular application is left to the preference of the operator.

The tubing is joined to the flow cell by means of a tubing connector. The connector is composed of three parts: the nut, the ferrule, and the body. The nut (made of Celcon®) is the same for either tubing, however, the ferrule and body differ depending on which tubing is to be used. The red body (made of Halar) and the smaller ferrule (made of polyethylene) are to be used with the $\frac{1}{8}$ inch tubing while the black body (made of Halar) with the longer ferrule (made of polyethylene) are to be used for the small 0.046 diameter tubing. The connectors are threaded and can be removed by unscrewing.

Note that there is a solid PTFE O-ring at the base of the threaded hole in the flow cell, which provides a seal between the fitting and the flow cell. When installing the fitting, great care should be taken so as not to cross-thread or overtighten the

fitting. It need only be hand tightened until the first significant resistance to motion is felt. The fitting should not be tightened with a wrench, as the threads in the flow cell may be damaged.

The tubing is installed into the fittings as shown in Figures 2-9 and 2-10. When using the 1/8 inch tubing and fitting, insert the tubing through the hole in the nut, place the ferrule on the tubing (approximately 1/4 inch from the end) and insert into the fitting body so that the tubing bottoms on the shoulder inside the fitting. The 0.046 diameter tubing is assembled the same way, except that the tubing is inserted flush with the end of the long neck of the ferrule (Figure 2-10). Carefully engage the nut on the fitting body and tighten the nut until the tubing resists rotation within the connection.

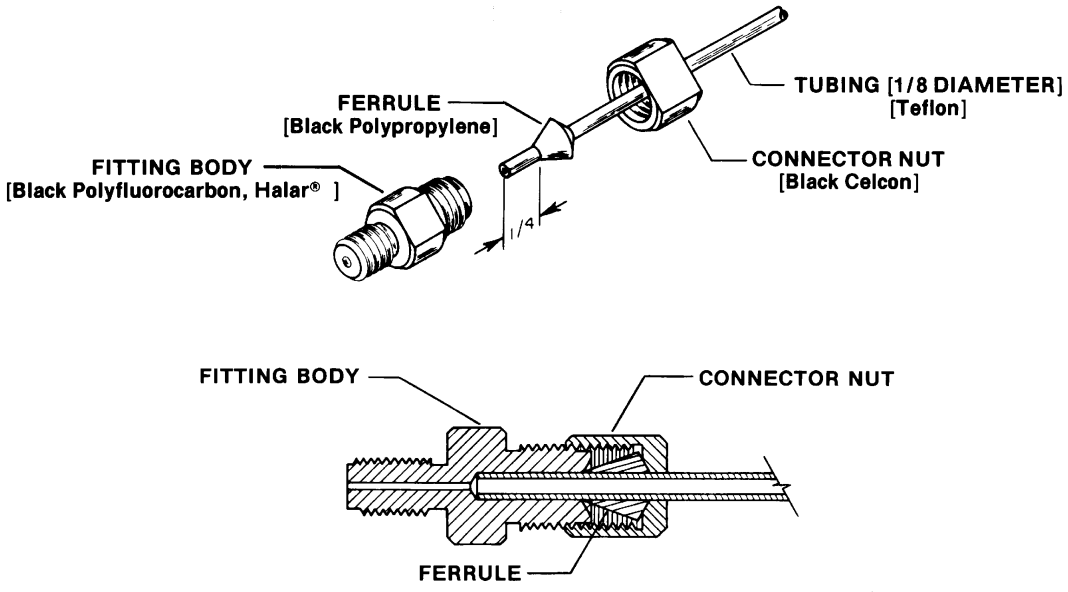


Figure 2-9 Tubing connector used with 1/8" tubing

The fitting body should be restrained from rotation during this operation by using a second wrench on the body shoulder. Caution should be exercised in engaging and tightening the nut so that the threaded portion of the parts are neither cross-threaded nor stripped. Also, overtightening the nut may damage the soft ferrule or deform the plastic tubing. However, some degree of deformation of plastic tubing in this type of seal is normal and is not harmful.

The quality of the seal is assured by the compression of the ferrule between the tubing and the conical section of the fitting body. Extra ferrules are available from Teledyne lsco.

The volume of liquid contained within the connecting tubing is quite small, thus minimizing the effects of laminar flow and gradient inversion upon the sample bands.

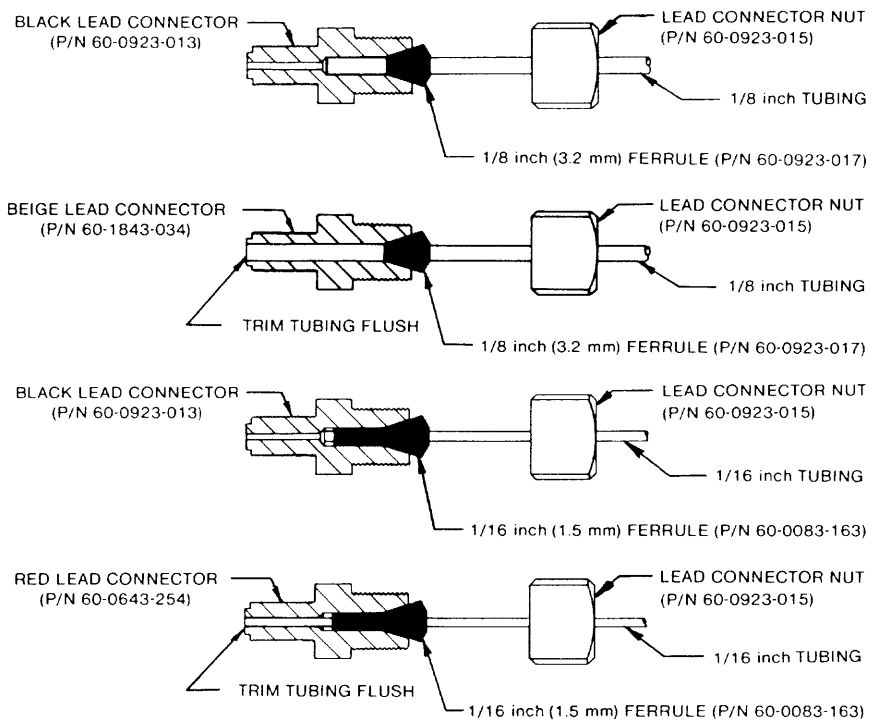
From the flow cell, the tubing is led to the discharge point of the fraction collector.

FERRULE APPLICATION INFORMATION

Two types of very low density polyethylene ferrules are provided for use with this instrument. Characteristic properties and application of these ferrules are as follows:

OD OF TUBING TO BE USED	1/16 inch (1.5 mm) with ferrule P/N 60-0083-163
	1/8 inch (3.2 mm) with ferrule P/N 60-0923-017
INSTALLATION PROCEDURE	Finger tightening of connector nut
COMPATIBLE SOLVENTS	Polyethylene ferrules may be used with acids, bases, and all organic solvents, but are subject to long-term damage due to swell when exposed to halogenated hydrocarbons over extended time. In most cases, such a damaged ferrule is still useable until the lead connector nut is loosened, unless the pressure of swelling collapses thin-walled connecting tubing.

FERRULES PICTURED AS INSTALLED



FERRULE INSTALLATION PROCEDURE

Slide lead connector nut over tubing. Slide ferrule over tubing. For black lead connector with either 1/16 or 1/8 OD tubing, insert tubing until it bottoms out on the lead connector. For red lead connector with 1/8 OD tubing, also insert tubing until it bottoms out. For red lead connector with 1/16 OD tubing or beige lead connector with 1/8 OD tubing, insert tubing through entire connector until it protrudes 1/8" beyond end of lead connector. Slide ferrule into lead connector and secure with lead connector nut. Trim excess tubing flush with end of lead connector. **CAUTION:** Do not overtighten lead connector nut! Overtightening can lead to collapsing thin-walled tubing.

1. Red lead connectors have 0.063" (1.5 mm) fluid passages.
   Black lead connectors have 0.036" (0.9 mm) fluid passages.

2. Tubing OD tolerance is ±.2 mm.

Figure 2-10 Tubing connector used with 1.5 mm or smaller tubing

2.9 Post Fractionating Procedures

After the contents of the centrifuge tube have been scanned and fractionated, as shown by the absorbance chart record, the pump should be shut off. The fractionated centrifuge tube may now be removed and replaced with another.

If this operation is performed in the following manner, there will be a minimum loss of chase solution and the chances of introducing air into the liquid system will be minimal.

Place the drive of the pump at a fairly fast speed (e.g., 10 to 20 ml/min.) in the REVERSE direction and turn it on.

The pump will begin to withdraw the chase solution from the centrifuge tube through the needle. At this point it may be desirable to introduce a small amount of distilled water into the fraction collector discharge spout to wash the previous solution from the flow cell (not recommended if Fluorinert is the chase solution). This is done by placing a low beaker of distilled water under the discharge spout and allowing several milliliters of water to be drawn into the flow cell by the pump.

Observe the meniscus as it travels down the centrifuge tube and stop the pump just before it reaches the holes in the needle, not the bottom of the tube. If all of the gradient has not been fractionated or if there is wash water floating above the chase solution, stop the pump just before the interface above the chase solution reaches the holes in the needle.

In this manner, most of the chase solution can be saved and reused. However, a small amount will be lost and it should be insured that enough chase solution remains in the pump to fractionate the next centrifuge tube. The liquid system should also be checked to make sure no air has been introduced.

After the centrifuge has been partially emptied, it may be removed. This is done by first loosening (not removing) the retaining nut on the flow cell to free the tube. Then, loosen the rear thumbscrew on the piercing apparatus and lower it, at the same time pulling the centrifuge tube out of the flow cell and holding it in contact with the bottom holder (Figure 2-11).

Figure 2-11 Removal of centrifuge tube from flow cell

The tube may now be removed and discarded. To minimize the spilling of the liquid left in the bottom of the tube, the tube and the bottom holder may be removed together.

After the fractionation of the final tube, the entire liquid system (pump, piercing assembly, flow cell, and fraction collector connecting tubing) should be thoroughly washed. This is most easily accomplished by removing the flow cell piercing assembly, etc., and washing them in hot water or in a mild detergent solution.

☑ Note

The flow cell windows do not normally need to be removed for cleaning.

The piercing system is removed by loosening the front thumbscrew and pulling the system straight forward out of its retaining slot. The bottom holder and threaded cap may then be removed for individual cleaning. If there has been any spillage of solution on the instrument itself, it should be cleaned off and not allowed to build up. If the holes in the piercing needle become clogged, they may be cleaned out with the piece of 22 gauge (0.029 inch diameter) wire supplied with the tube piercer.

2.10 Use of Tube Piercer with non-lsco Absorbance Detectors

The Tube Piercer may be used with absorbance detectors of a manufacturer other than lsco through the use of a "universal top holder" (part #68-0647-037). The lower portion of this top holder is identical to the lower portion of a density gradient flow cell. It differs from a flow cell in that the upper portion has been replaced with a fitting for 3 mm or 1.16 mm (0.046 inch diameter) OD tubing.

Position the flow cell of the alternative monitoring system directly above the top holder. Connect the flow cell to the top holder with small bore (approximately 1/2 mm) tubing having no changes in internal diameter or other irregularities that would disrupt a smooth flow of solution. Take care not to invert the gradient or disrupt the flow with improperly designed fittings.

2.11 Purification of Sucrose to Minimize Absorbance

Add decolorizing charcoal to a 60% solution of food grade sucrose in the ratio of 5 to 50 grams charcoal to 100 ml of solution. Boil gently for about five minutes and filter hot through a charcoal pad on a Buchner funnel.

The charcoal pad is best prepared by pouring a charcoal slurry over the filter paper in the funnel and then washing until the filtrate is clear. The pad should be from 1/16 to 1/8 inch thick. The sucrose solution must be hot as its viscosity is too high for rapid filtering at moderate temperature.

Usually the filtered sucrose solution will be increased in concentration after this process. Use a refractometer to determine the actual sucrose concentration before preparing the gradients.

2.12 Manual Formation of Gradients

Density gradients may be formed with automatic equipment such as the Isco Model 160 Gradient Former programmed by the Isco Foxy Jr. These items are provided with Isco's programmable Density Gradient System (part #67-9000-177).

Density gradients may also be made by several manual methods. Convenient protocol for layering gradient tubes with a constant buffer solution concentration throughout is described below. Make four gradients if three are to be used. This will provide a spare in case one is ruined by accident.

To make 25 ml (0.85 oz) linear gradients with 0.01M sodium citrate buffer throughout and with sugar concentrations from 400 g/l to 100 g/l, first dilute the stored 600 g/l sucrose in water with concentrated buffer. (To 50 ml 600 g/l sucrose solution, add 0.75 ml, 1.0M sodium citrate and enough water to make a volume to 75 ml).

After thorough mixing, pipet 7 ml into each centrifuge tube. To the residue of 47 ml of 400 g/l sucrose solution add 16 ml of 0.01 M sodium citrate. This will yield 300 g/l sucrose solution in 0.01M sodium citrate.

After thorough mixing, carefully pipet 7 ml of this solution into each centrifuge tube. Extreme care must be taken to layer this solution over the heavier solution already in the tubes. If layering does not occur, the tube must be discarded. Some people prefer a pipet with a bent tip or a hole in the side for this technique.

To the 35 ml (1.19 oz) of 300 g/l sucrose solution left in the stock preparation, add 17.5 ml (0.6 oz) of 0.01M sodium citrate, making a solution of 200 g/l 0.01 M sodium citrate. Carefully layer 7 ml of this solution to the top of the solutions already in the centrifuge tubes.

To the residue of stock solution (24.5 ml; 0.83 oz) add an equal volume of 0.01M citrate (24.5 ml; 0.83 oz) resulting in a 100 g/l sucrose solution in 0.01 M sodium citrate. Carefully layer 4 ml (0.14 oz) of this solution on top of the other solutions in each of the centrifuge tubes and carefully move them to a cold room or refrigerator for several hours (or overnight). The boundaries will diffuse and a good linear gradient will be formed, ready for use in the centrifuge.

Tube Piercer Installation Guide

Section 3 Maintenance

3.1 Routine Maintenance

The following section contains instructions for routine maintenance on various parts and systems, necessary from time to time for efficient performance of this equipment.

3.2 Cleaning the Tube Piercing System

The tube piercing assembly should be cleaned after each use before any spilled sucrose or salt has dried. If spilled solution is allowed to dry on the piercing apparatus, it will probably be necessary to disassemble the apparatus to clean it. This is done in the following manner:

1. Remove the tube piercing assembly by loosening the thumb screw that holds it in place.
2. Take the assembly apart by pulling off the bottom holder. Then hold the bottom cap in one hand, as shown below, and use a wrench to unscrew the Luer-Lok fitting.

Figure 3-1 Disassembling the tube piercing assembly

3. After the fitting is loosened and removed, the tube piercing assembly can be taken apart (Figures 3-3 and 3-4).

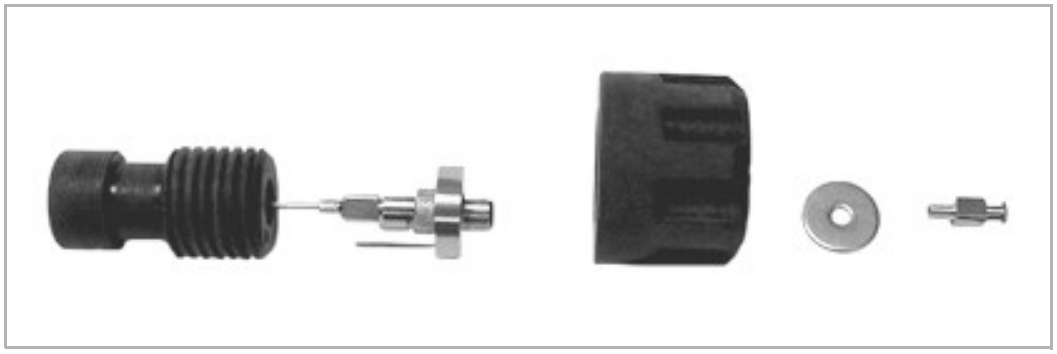


Figure 3-2 The tube piercing assembly, disassembled

4. Grasp the needle assembly with one hand, and use a wrench to unscrew the Luer-Lok fitting, as shown below.

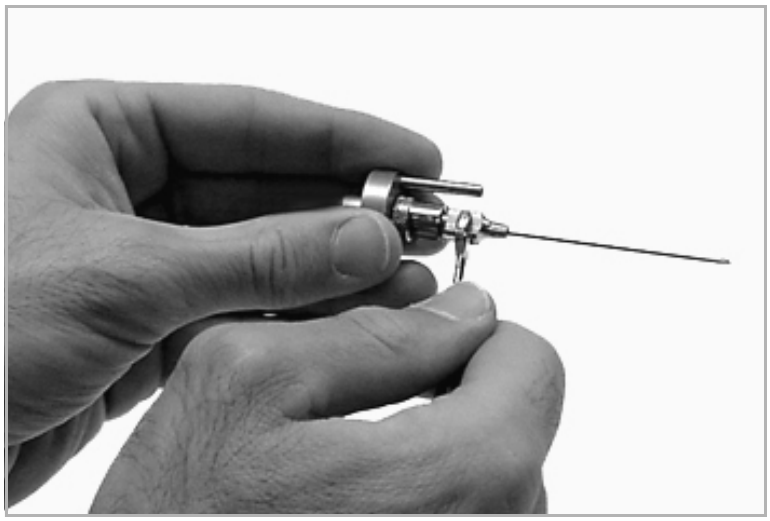


Figure 3-3 Disassembling the needle assembly

5. All of the individual pieces may now be cleaned by soaking them in hot water or a mild detergent solution.
6. Reassemble the needle and tube piercer assemblies by reversing the above steps.

3.3 Piercer Needle

The two holes in the piercer needle may occasionally become plugged with dried sucrose or salt solutions or with a small piece of centrifuge tube. If this occurs, remove the bottom holder to expose the needle holes. Then, poke a piece of 22 gauge (0.029 inch diameter) wire through the holes to clean them out.

After a period of time, it is possible that the needle may become bent or otherwise damaged and need replacing. If this is the case, remove the tube piercer assembly from the instrument and disas-

semble it and the needle assembly as described in Section 3.2. Additional needles are available from lsco (part #69-0643-164). Reassemble the tube piercer and reinstall on the instrument.

3.4 Flow Cell

If bubbles adhere to the flow cell, it is probably due to a dirty cell. Clean the flow cell by running detergent solution and then clean water through it. If the cell has been used with a sucrose solution, it may be necessary to either rinse it with alcohol after washing or to use an alkaline soap before the last water rinse. If necessary, the silica flow cell windows may be removed by unscrewing their slotted retaining rings, and then cleaning them.

If the flow cell leaks around the windows, there are two possible trouble spots:

1. Check the O-ring behind the retaining ring and replace it, if necessary, with one of the spares included with the flow cell.
2. If the O-ring is undamaged, reseal the windows with a small amount of stopcock grease between the window and flow cell.

⚠ CAUTION

Use grease sparingly, and be careful not to get grease on the area of the window that contains the light path of the flow cell, as it absorbs ultraviolet light. Also, grease within the flow cell may cause air bubbles to stick to the inner faces of the windows or the Delrin Cell.

⚠ CAUTION

Do not overtighten the retaining rings as flow cell threads may be stripped or the window cracked. Use great care in starting rings into threads, or they may be stripped and the flow cell ruined.

☑ *Note*

Extra windows have been included with the flow cell and are also available from Isco.

3.5 Bottom Holder Gasket (septum)

After repeated piercings, the rubber disk gasket (septum) in the bottom holder will wear out and will not provide a sufficient seal at the tube puncture area. If the septum is worn out, replace it with one of the spares included with the flow cell.

3.6 Service Department

If you have a problem with the instrument or need parts information, contact Teledyne Isco's Service Department toll-free at 800-775-2965, or e-mail IscoService@teledyne.com.

Please include all pertinent information that may be helpful in solving your problem. We strongly suggest that you contact the Service Department before deciding to return the unit for factory repair. Often, a problem can be solved with just a call or email.

3.6.1 How to Ship Returns

Be sure all parts and hardware are back in place before packing. Wrap the unit in heavy paper or put it in a plastic bag. If the original carton is not available, put the wrapped unit in a strong cardboard box at least six inches longer in each basic dimension than the unit. Fill the box equally around the unit with resilient packing material (shredded paper, bubble pack, expanded foam chunks, etc.). Seal it with strapping tape or gummed cloth tape and ship it to the address given previously.

It is very important that the shipment be well packaged and fully insured. Damage claims must be settled between you and the carrier. This can delay repair and return of the unit to you.

3.7 Purchasing Parts

Except for the Isco parts mentioned in this manual, you will need to contact Brandel to order replacement parts for the Tube Piercer.

The contact information for Brandel is:

Phone: 800-948-6506
E-mail: sales@brandel.com
Web site: www.brandel.com

The diagram on the next page (Figure 3-4) identifies the different parts of the Brandel Tube Piercer, to assist you in ordering parts.

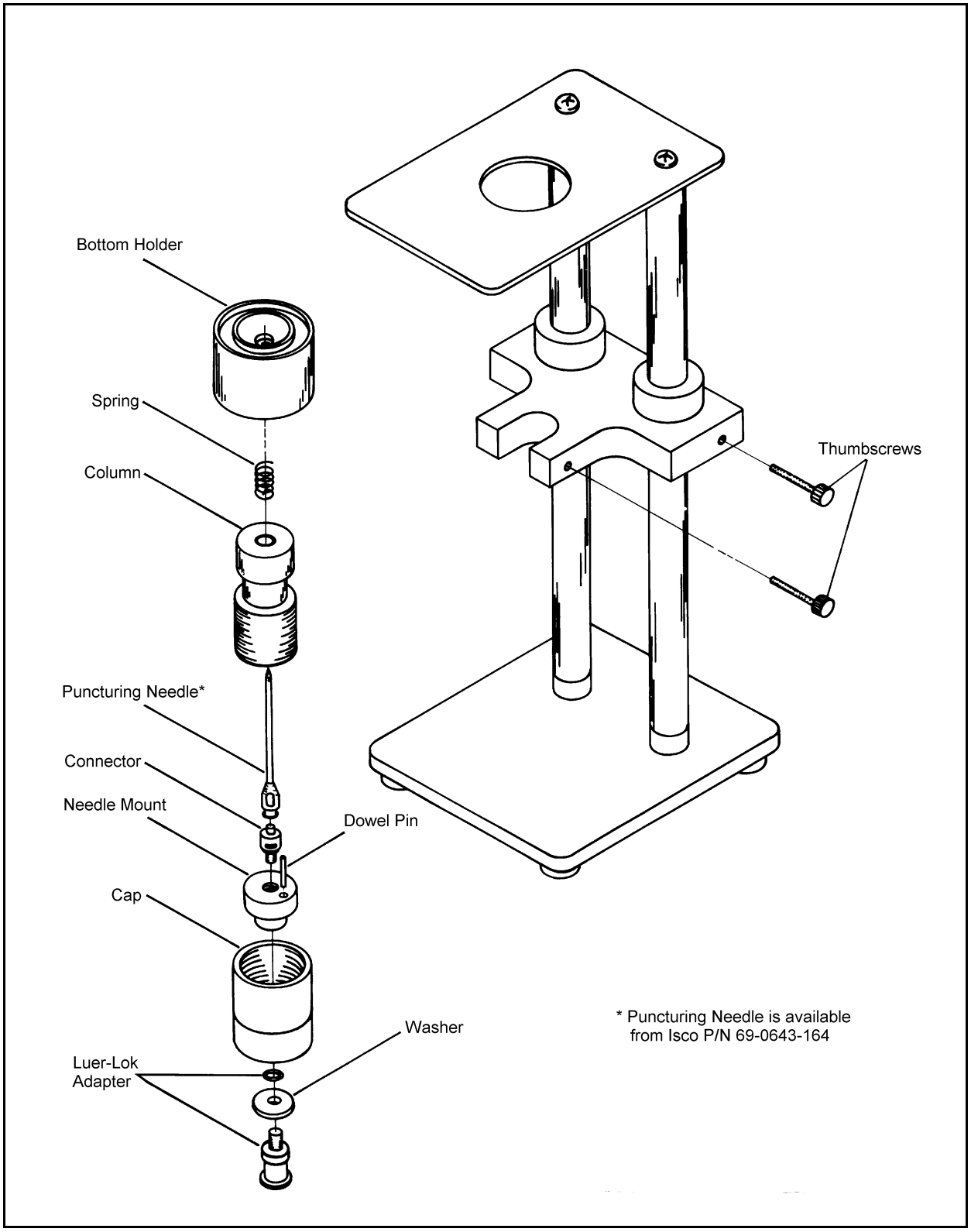


Figure 3-4 Tube piercer diagram