Lab Safety Tips

Economical Apparatus for Safe, Accurate Recovery of Biohazardous or Radioactive Gradient Fractions

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Density gradients are used extensively for the separation and purification of various molecules. The collection or fractionation of gradients is commonly accomplished in one of two ways: by puncturing the tube at the bottom and draining the contents or by displacing the gradient from the top (1).

Without the use of a specialized fraction collector, which can be expensive and bulky, puncturing the tube and draining can be difficult due to the inability to control flow rate. This results in inconsistent fraction volumes, and the loss of sample while puncturing or moving from tube to tube. Puncturing the tube free-handedly is also hazardous when the gradient contains radioactive or infectious material. Collection from the top of the gradient can be difficult as well. Attempting to collect fractions from precisely the top of the meniscus is often awkward, and can result in aspirating air bubbles, or aspirating from below the meniscus, both of which disturb the integrity of the gradient.

Figure 1. A gradient collection system. A) The configuration of the gradient tube holder; (i) gradient tube, (ii) syringe barrel, (iii) needle holder, (iv) threaded needle with Luer-Lok adapter and (v) Luer-Lok blunt needle connected to (vi) tubing, which leads to the peristaltic pump. B) Schematic diagram of collection system. Gradient tube is placed in tube holder (A) where it is punctured. The gradient flows down through the needle and tubing to the peristaltic pump, where its flow rate is controlled and is dispensed as desired.

We have designed an apparatus for gradient recovery that allows for precise fraction collection from 14-mm tubes (No. 344060; Beckman Instruments, Fullerton, CA, USA) without these difficulties (Figure 1). A needle holder (Vacutainer No. 364893; Becton Dickinson, Rutherford, NJ, USA) is mounted by clamp to a support stand. The threaded needle of a blood collection set (Vacutainer No. 4919) is screwed into the needle holder from the bottom, and a 12-mL syringe barrel (Monoject No. 512910; Sherwood Medical, St. Louis, MO, USA) is placed into the needle holder from the top (Figure 1A). The syringe barrel acts as a spacer, controlling the distance that the Vacutainer needle enters the tube. A blunt-end needle with a Luer-Lok® hub (Monoject No. 202355; Sherwood Medical) is attached to the Luer-Lok adapter of the threaded needle and connected to tubing (No. S-54-HL; Norton Performance Plastics, Akron, OH, USA) that leads to a peristaltic pump (Model P-1; Pharmacia Biotech, Uppsala, Sweden). The gradient tube is inserted into the syringe barrel and carefully pushed down onto the needle until punctured. The gradient flows down through the needle and tubing, as its flow rate is controlled by the peristaltic pump, and aliquoted accordingly (Figure 1B).

We have found several advantages using this setup. First, the use of the peristaltic pump allows for the fine control of the flow rate, resulting in accurate, consistent fractionation. Since the flow is controlled by the speed of the pump, one can increase or decrease the flow rate depending on variables such as fraction volume desired or to facilitate movement between collection tubes. The steadiness of the flow also maintains the integrity of the gradient during recovery. Second, the setup of the gradient tube holder (Figure 1A) allows for safe, effective puncture of the gradient tube. Since the gradient tube is housed inside a syringe barrel, inside a needle holder, the gradient tube and needle are stable during the puncturing step and remain stable throughout recovery. Any risks of exposure when handling radioactive or infectious substances are greatly reduced due to the placement of the needle used for

puncturing and the blunt end of the connector needle. Moreover, all of the materials in the gradient tube holder setup are disposable. No disassembly of parts is necessary, further reducing the risk of needle prick. The setup can simply be discarded altogether after removing the connection to the pump. Disinfecting agents may also be run through the system before disconnection, if necessary. Third, the setup is easily assembled and inexpensive, compared to elaborate fractioning systems, which can cost more than \$2,000, not including tubes and other disposables. A single setup for this system, including everything but the peristaltic pump, costs less than \$3. The pump we use is Model P-1 from Pharmacia Biotech and costs \$995. Other peristaltic pumps are available at a lesser cost (Easy-Load 150V, \$660; Millipore, Bedford, MA, USA).

REFERENCE

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