spINSIGHTS

from the lab

In this issue: How can I minimize potential sample waste when using the OptiMATE Gradient Maker to dispense gradients?

The OptiMATE Gradient Maker uses automation to dispense **step** and **continuous density gradients** (or combinations of the two) for a range of ultracentrifuge tube types with ease, precision and accuracy.

The instrument can incorporate samples to be purified along with buffers and/or diluents with the gradient modifier solutions (e.g., cesium chloride, iodixanol, sucrose) to generate ultracentrifuge-ready density gradient tubes.

To sustain accuracy and effectively dispense gradients, the OptiMATE Gradient Maker requires steps that are not part of a manual density gradient dispense process.

Because density gradient purification samples may be precious, concerns about sample loss from these steps can be addressed in a straightforward manner.

Non-dispense steps and how to minimize sample loss:

Before dispensing density gradient samples into tubes, the OptiMATE Gradient Maker includes priming and calibration steps.

1. Priming

This step enables users to fill instrument tubing with the connected reagent/sample, which is important to minimize air bubbles in the density gradient generation process.

After the entire tubing, manifold and dispense probe volume is filled, the fluid starts dropping into a prime tube used for waste collection. When priming tubing with a sample, the easiest way to minimize sample loss is to:

- Prime the sample tubing separately
- Abort the process as soon as drops fall into the prime tube

Done correctly, this can minimize sample loss to < 100 µL.

Alternatively, users can prime tubing with sample-free buffer or media instead of the sample, and "hot-swap" the Luer connection to the sample at the time of dispensing to ultracentrifuge tubes.

This minimizes loss of the few drops of sample from priming, but will dilute the sample by the volume of the tubing, which may not be acceptable, depending on the sample volume to be dispensed in each tube.

2. Calibration

This step dispenses 1.11 mL of each chosen fluid—with an additional dispense (0.5 mL) of the least dense fluid—into the instrument's calibration tube, and measures liquid level in the tube to compensate for variation in the fluidic tubing and clamping.

While calibration can be skipped to avoid sample loss, if the instrument is being used with no changes to the fluidics after a recent calibration, it's a useful step for maintaining dispense precision and accuracy.

Sample-free buffer/media can be used for the calibration process (instead of the sample), if they have the same density as the sample-containing version. A prime step can be repeated after the calibration to fill the tubing with the sample (using the loss minimization strategy described for priming).

Recovering sample in tubing after the density gradient dispense

If adequate sample is available in the stock container, instrument tubing should contain the sample after the density gradient dispense. Opening the pump's clamp allows sample in the tubing to flow back into the stock container.

Alternatively, a syringe can be used to recover the sample in the tubing at the Luer connection to recover the sample separately.

If low sample volume is available (e.g., just enough for 1 ultracentrifuge tube), the sample can be incorporated as a manual layer separately, while the density gradient can be dispensed by the OptiMATE Gradient Maker.

Summary

Most automated liquid dispense methods will include challenges with effective sample management and loss minimization that need to be managed via fine-tuning the dispense workflow. Using the strategies described above can minimize sample loss to < 300 µL while sustaining the effectiveness and benefits of using the OptiMATE Gradient Maker.

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2025-GBL-EN-109022-v1

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