APPLICATION NOTE

How to Easily Create Accurate Step Gradients Using OptiMATE Iodixanol Reagent

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OptiMATE Gradient Maker

OptiMATE Iodixanol

Iodixanol (IDX)-based density gradient purification is a long-standing method for purifying viral vectors because it is largely independent of any variations made to the product.[1] IDX is non-ionic and mostly non-reactive, which allows for its use with a variety of products with little concern about toxicity or modification of the product[1,2]

A 15-60% w/v IDX step gradient enables effective enrichment of full adeno-associated virus (AAV) capsids in a short 2-hour ultracentrifugation time.[3]

In this application brief, we describe how to use OptiMATE 80% w/v IDX reagent as a cost-effective alternative to 60% w/v IDX solutions. These tools and processes are designed to streamline a transition of your existing IDX protocols to OptiMATE technologies, enabling hassle-free density gradient workflows.

Methods

OptiMATE IDX solution

The Intellifuge tool provides a free, easy-to-use calculator (Figure 1) to help users of 60% w/v IDX solutions seamlessly transition to OptiMATE 80% w/v IDX reagent. This calculator determines the volume of 80% w/v IDX for use in place of specified volumes of 60% w/v IDX and sample/buffer used to create each layer.

Concentration of IDX in the layer to be achieved ($[IDX]$) can be derived from the volume of the 60% w/v IDX (v_{60}) and sample/buffer (v_s) used:

$$[IDX] = \frac{v_{60}}{v_{60} + v_s}$$

Using the concentration identified, the volume of OptiMATE 80% w/v IDX (v_{80}) to be used to achieve a layer of specified volume (v_t) is calculated based on molar conservation:

$$v_{80} = \frac{80}{[IDX] \times v_t}$$

...Where the value 80 comes from the % w/v concentration of the OptiMATE IDX reagent. Using this, the volume of sample/buffer to be added to form the layer (v_s') can be calculated by:

$$v_s' = v_t - v_{80}$$

When using the OptiMATE Gradient Maker to make these density gradients, values are automatically calculated by the system based on inputs of stock solution concentration and density, and the desired concentration, density or volume.

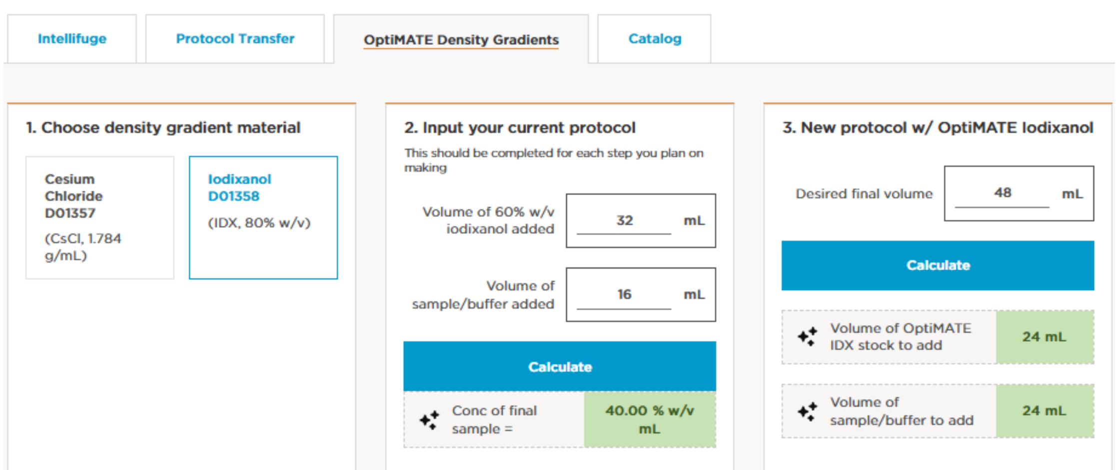

Figure 1: The OptiMATE Density Gradients interface in Intellifuge to help calculate IDX concentration and OptiMATE IDX reagent to be used.

Density gradient profile comparison

The OptiMATE IDX reagent has a higher density (1.42 g/mL) than 60% w/v IDX solutions (1.32 g/mL), which also increases viscosity. The OptiMATE Gradient Maker is designed to ease the labor-intensive and error-prone nature of IDX step gradient generation as described in previous studies.[4]

However, the OptiMATE IDX reagent can still be manually dispensed into ultracentrifuge tubes with minimal difference from dispensing an IDX step gradient prepared from a 60% IDX solution.

Table 1 describes a typical IDX step gradient used for the purification of AAV dispensed into a 39 mL Quick-Seal tube. Stock solutions of 15% (containing 1M NaCl), 25%, 40% and 60% IDX were prepared from the OptiMATE IDX reagent and a 60% IDX solution. The volumes specified in table 1 were then dispensed into 39 mL Quick-Seal tubes, which were then punctured from the bottom and fractionated.

Density of these fractions was determined to compare the density gradient profile of the tubes for differences. This density gradient profile is shown in Figure 2. There is good overlap between the two gradient profiles despite some error that could result from the manual fractionation process.

Iodixanol %	Tube volume (mL)
Total	39
15% (with 1M NaCl)	8
25%	6
40%	8
60%	5
Water	12

Table 1: Tube composition for IDX step gradient comparison.

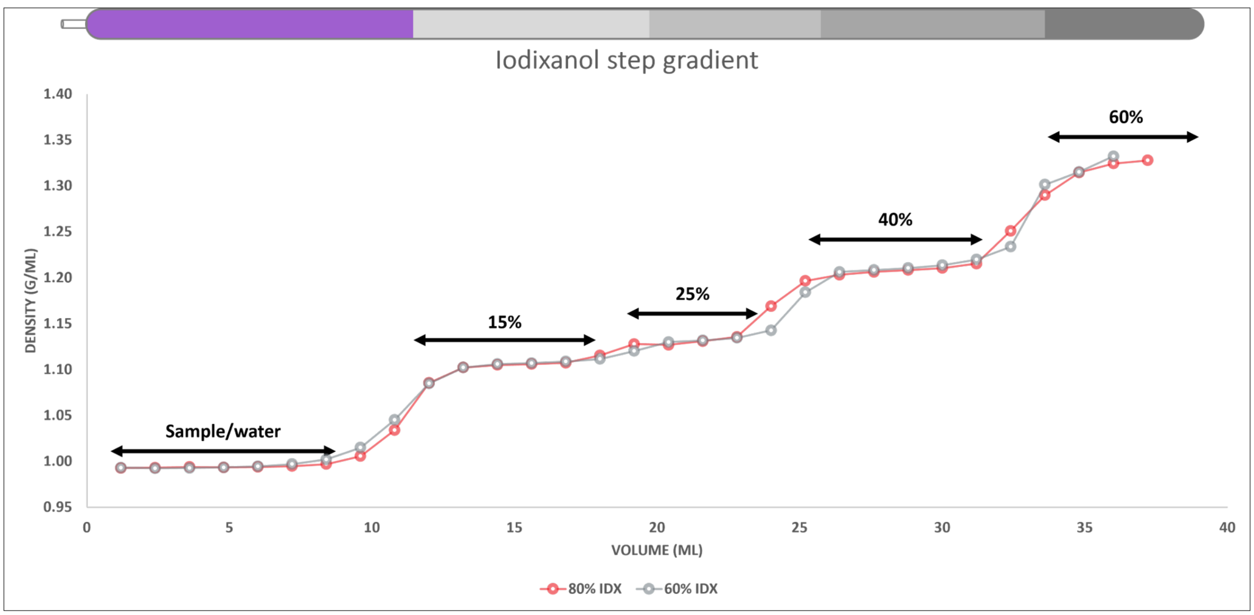

Figure 2: Density gradient profile comparison of IDX step gradients generated with 60% IDX solution and OptiMATE 80% IDX reagent.

Conclusions

The OptiMATE IDX reagent can serve as a viable equivalent for existing 60% w/v IDX solutions. It also offers higher density (1.42 g/mL) due to its 80% w/v concentration, which makes it accessible to density gradient experiments that were previously limited because of the lower density of 60% IDX solutions.

References

1. El Andari, Jihad, and Dirk Grimm. "Production, Processing, and Characterization of Synthetic AAV Gene Therapy Vectors." Biotechnology Journal, vol. 16, no. 1, Jan. 2021, p. 2000025
2. Lam, Anh K., et al. "Comprehensive Comparison of AAV Purification Methods: Iodixanol Gradient Centrifugation vs. Immuno-Affinity Chromatography." Advances in Cell and Gene Therapy, vol. 2023, 2023, p. 2339702
3. Zolotukhin, S., et al. "Recombinant Adeno-Associated Virus Purification Using Novel Methods Improves Infectious Titer and Yield." Gene Therapy, vol. 6, no. 6, June 1999, pp. 973–85
4. Venkatakrishnan and Sternisha. Application note: Reducing Variability and Hands-On time in Viral Vector purification using the OptiMATE Gradient Maker.

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