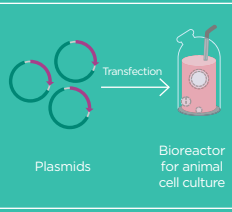
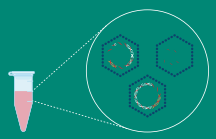
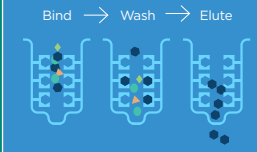
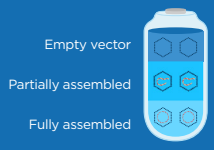
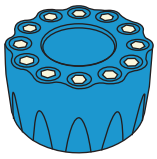
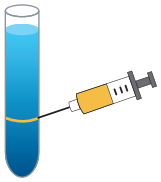
Viral Vector Purification with Ultracentrifugation:
How do I separate empty, partial & filled viral capsids?

BECKMAN COULTER Life Sciences

Vectors such as AAV and adenovirus are powerful delivery tools that are currently used in research, preclinical, and clinical developments. Density gradient ultracentrifugation (DGUC) offers a serotype-independent method of separating empty, intermediate, and full viral particles.

WORKFLOW

Cell culture + transfection	Clarification + concentration	Viral Separation	Vector Purification	Polish + Sterile Filter
				
Create cell lines producing viral vectors for purification	Separate cells from culture media; enrich for virus particles	Separate viruses from residual impurities	Separate fully loaded viral capsids from empty + partial capsids	Polish and prepare therapeutically effective viral vectors (remove remaining impurities while concentrating viral capsids)

1. DENSITY GRADIENT FORMATION	2. LOAD & RUN ROTOR	3. SAMPLE RECOVERY
		
• Choose a gradient method • Choose a gradient material	• Choose a tube type • Choose a rotor type	• Choose whether to collect all fractions or syringe extract only what is needed

Use Density Gradient Ultracentrifugation to achieve >99% full viral capsids*

* See doi: 10.1089/hgtb.2015.051 for an example protocol with ultracentrifugation

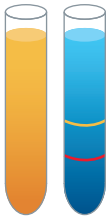
Considerations in Density Gradient Ultracentrifugation

1. DENSITY GRADIENT FORMATION

GRADIENT METHOD

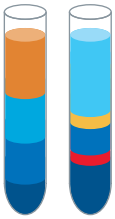
Isopycnic:

- Materials separate based on buoyant density in a self-forming density gradient that results in a continuous density gradient
- Ideal for separating same size or mass but different buoyant density materials (e.g., empty vs loaded virus)
- Highest resolution because density gradient is a continuum



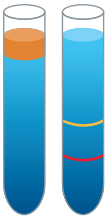
Equilibrium zonal:

- Materials separate by density in a pre-formed density gradient that does not achieve a continuous gradient, but rather has discrete segments of different densities
- Provides a balance of speed (because the gradient is pre-formed) + purity
- Less maximum resolving power than isopycnic



Rate zonal:

- Materials separate based on sedimentation coefficient (e.g., including size and mass) in a pre-formed density gradient
- Ideal for separating a protein complex from other proteins



GRADIENT MATERIAL

Iodixanol

- Typically faster protocols
- Minimal interference with subsequent analysis / analytics
- Typically used for equilibrium zonal gradients
- Iodixanol equilibrium zonal density gradients are often selected for rapid, high throughput purification of vectors in discovery or development

Cesium Chloride (CsCl)

- Better purity and resolution of empty, full, and partial capsids
- Typically more time-consuming protocol
- May require lengthier buffer exchange prior to analysis
- Typically used for isopycnic gradients
- CsCl isopycnic density gradients are often selected for the highest purity results in manufacturing or when producing reference standards

2. LOAD + RUN ROTOR

TUBE SEALING METHOD

OptiSeal

- Plug-based seal
- Faster to seal
- No physical guarantee of tube remaining sealed during transport / handling

Quick-Seal

- Permanent, guaranteed seal
- Heat-based seal
- Slower to seal

TUBE MATERIAL

Ultra-Clear

- Highly transparent tubes, easier to see bands
- Not autoclavable, but can be cold sterilized

Polypropylene

- Resistant to more chemicals than Ultra-Clear tubes
- Autoclavable
- Available for Optiseal and Quick-Seal tubes

Sterile + Certified Free* Tubes

- Available for both Ultra-Clear and polypropylene tubes
- Only Quick-Seal and open top tube types available

*Based on sample results below detectable limits

2. LOAD + RUN ROTOR (Continue)

ROTOR TYPE

Vertical:

- Most efficient rotor for (a) equilibrium zonal separations and (b) isopycnic separations with minimal contaminants that pellet

Near Vertical:

- Ideal for isopycnic separations with crude sample containing significant amounts of contaminant

Fixed Angle:

- Ideal for labs either budget- or space-constrained requiring 1 rotor suitable for both density gradient and pellet separations

Swinging bucket:

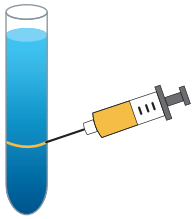
- Ideal for rate zonal separations
- Inefficient for other density gradients (e.g., viral separation)

3. RECOVER SAMPLE

SAMPLE RECOVERY

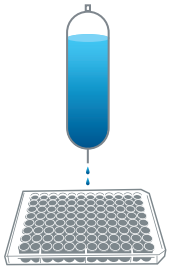
Syringe extraction:

- Precise method to remove only the band of piercing the tube with a syringe to collect the band
- Ideal for well-developed processes where the desired band of interest is known visually or by location.



Fractionation (from middle or bottom of tube):

- Pierce tube with a needle to drain out some of all of the solution in small increments, allowing independent analysis of each fraction.
- Ideal when bands are not visible or when seeking to characterize the composition of multiple bands



BECKMAN COULTER Life Sciences

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com
22.04.4643.CENT