BECKMAN COULTER Life Sciences

Getting started with Density Gradient Equilibrium Analytical Ultracentrifugation

Simple analysis, easy interpretation

AUC enables many different experiment types, each with its own strengths. Sedimentation Velocity (SV-AUC) as been cited as the gold standard[1] for AAV empty/full characterization and is frequently used for other applications as well. For those interested in using less sample than required in SV-AUC, analytical band centrifugation can be an option.[2] A third orthogonal technique is Density Gradient Equilibrium (DGE). DGE experiments utilize an easy-to-analyze method that characterizes empty/full viral capsids with significantly less sample requirements than other methods. Some advantages of DGE are listed below:

- **More straightforward**
  - Intuitive interpretation, no special AUC software or supercomputers needed
- **Less sample**
  - DGE-AUC can achieve 30X greater sensitivity
- **Higher throughput**
  - Can use the 6-sector centerpieces, resulting in 3x sample throughput
- **More relatable**
  - DGE-AUC is analogous to industry-standard CsCl prep gradients
- **Faster optimization**
  - Multi-parameter optimization screen achievable in a single experiment
- **More sample types**
  - Serotype agnostic; not size-limited; works well for AAV, adeno, plasmids

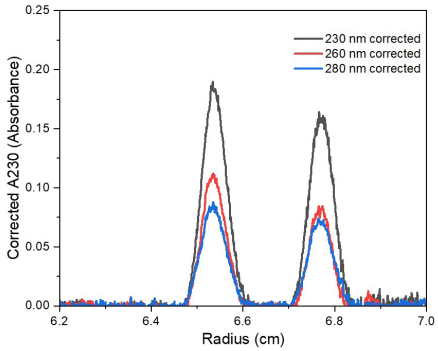


Figure 1. Example data output of a DGE-experiment. Empty capsids are in the left peak; full capsids in the right.

- **More data**
  - DGE-AUC is orthogonal to well-established SV-AUC methods
- **Higher resolution**
  - DGE-AUC is not time-resolved, thus multiwavelength experiments still have high resolution; scan replicates can also be averaged for higher accuracy
- **More tunability**
  - You can tune throughput, sensitivity, and resolution with Centerpiece; can also adjust volume, rpm, density, temp
- **More tolerant**
  - DGE-AUC is tolerant of its common buffer components, including stabilizers like pluronics, sucrose

When do I prefer SV data?

If you require high-resolution aggregate findings, you should use SV-AUC. With the addition of DGE-AUC in your arsenal, you have an orthogonal technique with the same instrument.

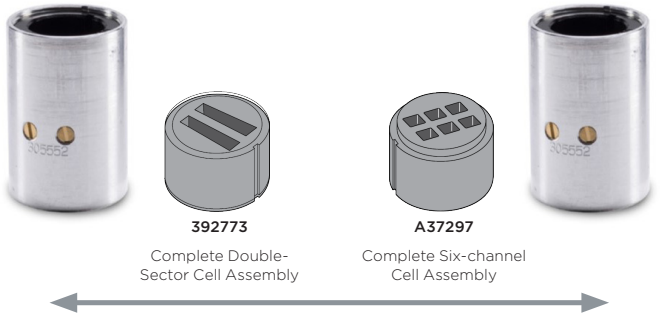
1. See DOI: 10.1089/hgtb.2015.048. For more information, read **Assessing the Quality of Adeno-Associated Virus Gene Therapy**
2. See DOI 10.1016/j.omtm.2021.04.008

Density Gradient Equilibrium Analytical Ultracentrifugation

When getting started, you will want to test various parameters to understand what works best for your samples (e.g., various sample concentrations with various densities).

Reagent and Sample Preparation

1. Prepare a high-density (≥ 1.7 g/mL) stock solution of optical grade CsCl. Use a refractometer to measure refractive index (RI) and verify density of the stock solution and any subsequent dilutions.
    - Refer to page 7-6 in **Beckman Coulter's ultracentrifuge catalog** for an RI to density conversion table for CsCl.
2. Prepare cell assemblies. Double Sector and Six-channel Epon-charcoal centerpieces with sapphire windows are preferred.
    - Find video guidance at **becls.co/auc-cell-assembly**
3. Prepare sample solution to the desired density. To do so, you must solve two equations (where buffer = buffer + H_2O + Sample):
    - *Target Density x Required mLs = Stock CsCl Density x Volume Cscl + Buffer Density x Volume Buffer*
    - *Volume CsCl + Volume Buffer=Required mLs*



For example, if you require 1mL of 1.35 g/mL CsCl, and your starting stock of CsCl is 1.722 g/mL and your buffer density is 1.01 g/mL, then you would solve the following:

- $1.35 \times 1\,mL = 1.722 \times V_{CsCl} + 1.01 \times V_{buffer}$
- $V_{CsCl} + V_{buffer} = 1\,mL$

Solving those equations yields 478 µL of CsCl stock and 522 µL of sample, buffer, and H_2O.

- Assuming that the buffer is 20x PBS stock, the virus stock absorbance is 5 OD, and the desired OD is .5, the final volumes would be: 100 µL virus stock @ OD = 5.0, 50 µL PBS stock at 20x, and 372 µL H_2O

4. Load the sample and reference into the appropriate sectors in the cell assemblies and seal them. Notably, the volume in each sector should be equal to ensure gradient matching in the sample and reference sectors.

Experiment Setup

A major advantage of DGE-AUC is the ability to test many parameters in a single experiment, which allows for rapid method optimization. The primary parameters to test include speed (rpm), solution density, and sample/particle concentration. High speed gives the greatest sensitivity and broadest range of densities to assess, while lower speed gives a narrower range and greater resolution.

1. Select starting density. Typically, this will be close to the buoyant density of your particles of interest.
2. Select a high-low absorbance range.
3. Create method scan.

See the following page for example parameters and run conditions for a first time DGE experiment. Multiple different densities, speeds, and sample volumes are suggested to test many parameters at once.

	Sample sector			Reference sector	
Cell	Sample Absorbance (A_{260})	Solution Density (g/mL)	Total volume (µL)	Composition	Volume (µL)
1	0.2	1.33	420	Buffer + CsCl	420
2	1.0				
3	0.2	1.35			
4	1.0				
5	0.2	1.37			
6	1.0				
7	--	1.35		Buffer only	
8	Counterbalance				

Table 1. Example of Double Sector cell setup for screening adenovirus.

			Absorbance			Interference		
Stage	Temp	Speed (krpm)	Scan Count	Scan Freq (m)	Wave-lengths	Scan Count	Scan Freq (m)	Time (h)*
1	20° C (Or 4° C)	0	Optional stage for temp equilibration					2
2		42	96	10	230, 260, 280	96	10	16
3		40	48			48		8
4		35	48			48		8
5		30	48			48		8
6		25	48			48		8

Table 2. Example of method scan creation for screening adenovirus.

*When first getting started, longer runs can ensure you reach equilibrium and test multiple different speeds. You can then review the data to find when equilibrium was reached, and what speeds you would prefer to analyze to significantly shorten your run time.

6-sector data analysis

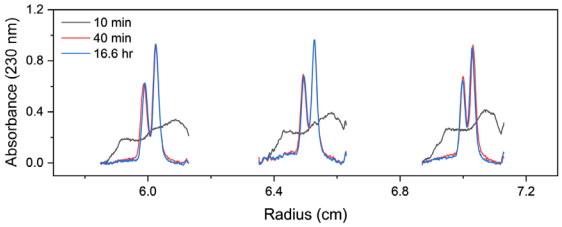


Figure 2. Empty + full adenovirus at 1.3496 g/mL and a starting absorbance of .4 at 260nm.

The above results show that after optimizing density and absorbance levels equilibrium with adenovirus was successfully reached in 40 minutes (1.3496 g/mL and starting absorbance of .4 at 260nm). Once you have determined your necessary parameters, your ensuing experiments can be 1 or 2 stages to get the data needed, and can be faster than 1 hour in length.

Experiment Execution

1. Balance the cells appropriately then load the rotors and start the experiment using both interference and absorbance.

2. Use the live data to monitor the equilibration process.

3. Analyze the data in standard data analysis and graphing software such as Microsoft Excel or Origin.
   - Download an example DGE-AUC Origin walk through at **becls.co/dge-analysis**

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