

Snag spot-on adenovirus titer & characterization with Stunner

Introduction

Adenoviral vectors (AdV) are extremely versatile – capable of vaccinating against viruses, delivering therapeutic genes and directly battling cancer as an oncolytic vector. Getting quick answers on the capsid titer and the empty/full ratio of AdV is a big deal, since titering methods for AdV can take hours or days, and empty capsids are the most common junk targeted by purification^{1,2}.

Traditional analytical methods for AdVs are generally slow, costly, labor-intensive and deliver only one piece of data at a time. Even the fastest method out there – measuring UV/Vis absorbance at 260 nm (A260) – still sucks up time as you need to boil samples in SDS and cool them off¹. In the literature the A260 assay has a classic rule that says $1\text{AU}_{260\text{nm}} = 1.1\text{E}12 \text{ vg/mL}^3$, but the accuracy of that conversion has been questioned by others who have proposed $1\text{AU}_{260\text{nm}} = 1.2\text{E}12^4$ or $1.8\text{E}12^5 \text{ vg/mL}$.

There are many more tools to check your AdV, but most of them take a bunch of time and expensive reagents – or need to be tuned to work for your specific serotype or transgene. Quantitative polymerase chain reaction (qPCR) is well-known for sequence-specific DNA quantification, but takes hours, tons of steps, and tells nothing about empty capsid titer. Electron microscopy (EM) along with anion-exchange high performance liquid chromatography (AEX-HPLC) both yield empty and full capsid titers. Yet, EM is prone to subjective data interpretation and AEX-HPLC needs to be optimized for specific samples and only runs samples one-by-one⁶⁻⁹.

Stunner is a one-of-a-kind platform that combines high-speed UV/Vis spectroscopy with static and dynamic light scattering (SLS & DLS) to measure titers down to $1\text{E}9 \text{ cp/mL}$, get a read on empty/full ratio, and check size and aggregation (Figure 1). Stunner reads samples without any sample prep, dilutions,



Figure 1: Stunner combines UV/Vis, dynamic and static light scattering (DLS and SLS) to provide accurate and precise characterization of biologic and gene therapy samples.

standard curves, labels or dyes. It is not serotype specific, only needs 2 μL of sample and reads up to 96 samples in 1 hour. For higher throughput, it's fully automatable and can be equipped with 21 CFR Part 11 software tools.

Stunner's Adeno Quant software application brings together DLS, SLS and UV/Vis to deliver spot-on capsid titers, empty/full ratio, plus total protein and DNA quantification (Figure 2). DLS is a powerful technique that identifies the size of particles and their distribution in a sample. The DLS intensity distribution shows the relative intensities of light scattered by capsids, aggregates, and any smaller particles in a sample. Gathered during a DLS experiment, SLS intensity is directly proportional to particle concentration, but full and empty capsids scatter different amounts of light due to their different compositions. So, for a given intensity of light scattered from a sample, you need more than just DLS and SLS to get an accurate particle concentration – you also need empty/full ratio data too. UV/Vis allows Stunner to determine total protein and DNA amounts in a sample, and leads to the empty/full ratio. When UV/Vis is combined with DLS & SLS data, Stunner has all the puzzle

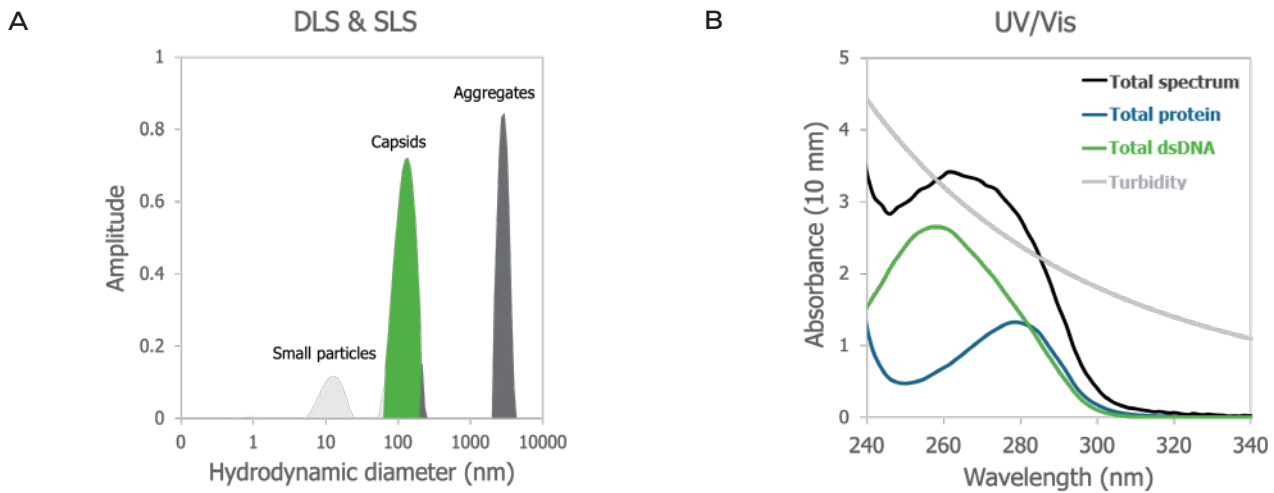


Figure 2: Stunner is the only instrument that combines three technologies to determine adenovirus capsid titer, empty/full ratio, and more. The DLS intensity distribution reveals intact capsids (green peak), as well as small particles (light gray peak) and aggregates (dark gray peak) (A). UV/Vis measurements automatically have sample turbidity removed (light gray line), to show the remaining total absorbance (black line), that is broken down into the amount of dsDNA (green line) and protein (blue line) (B).

pieces it needs to calculate total capsid titer, full capsid titer, and the empty/full ratio of an AdV sample (Figure 3).

Methods

Three lots of CsCl-purified Adenovirus 5 vectors from 3 vendors (Vendor 1, 2, 3) were stored at -70°C . To avoid freeze/thaw cycles, they were thawed once before first measurement and kept at 4°C in between experiments that were performed within 4 days.

The Adeno Quant application was selected in Stunner Client using 4 DLS acquisitions of 5 seconds

each with a water blank. Stunner measures the full UV/Vis absorbance spectrum and determines protein and dsDNA titers based on built-in algorithms for molar extinction coefficients and differentiating the absorbance contributions of protein, dsDNA, and common impurities in AdV samples. Molar extinction coefficients for each AdV were calculated based on published amino acid sequences. Molar extinction coefficients for each viral genome were calculated based on the DNA size in bases (b) provided by the manufacturer. Total capsid titer, full capsid titer, dsDNA titer, protein titer, % empty, % full, Z-average size and PDI were determined by the Adeno Quant application in Stunner Analysis.

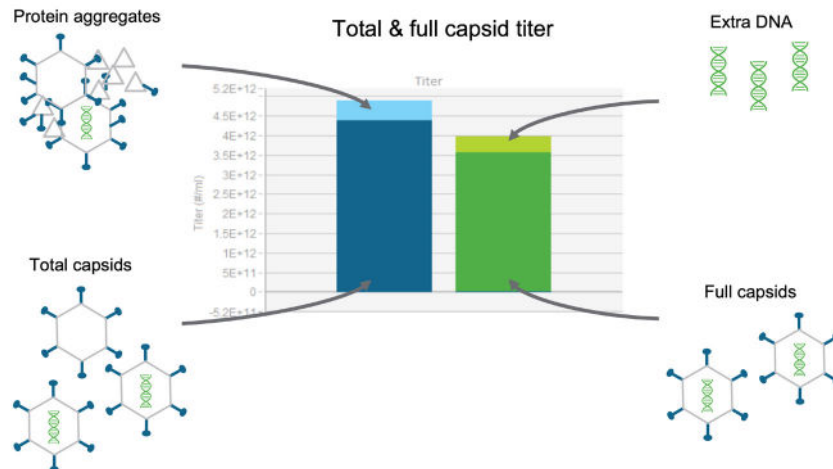


Figure 3: Stunner determines the number of total capsids (dark blue), the amount of free & aggregated proteins (light blue), full capsid titer (dark green), and free & aggregated dsDNA (light green).

Results

A260 and so much more

There's no doubt that UV/Vis spectroscopy is one of the fastest analytical techniques out there for a read on AdV sample titer. However, AdV particles are so large that they scatter lots of light and the resulting turbidity makes classic A260 readings very variable. Denaturing everything with SDS and heat breaks down the particles and removes scattering so UV/Vis readings are more reproducible. However, the extra steps hamstring the simplicity and speed of UV/Vis⁴, turning a fast assay into a 30 minute chore. Stunner's Adeno Quant application lets you measure your adenoviral vector as is, without any sample denaturation. Advanced Unmix algorithms analyze the total UV/Vis absorbance from AdV samples and divide it into what's coming from turbidity (AdV light scattering) and the actual absorbance by your adenoviral vectors. Eliminating turbidity mathematically means Stunner gets an A260 result in seconds, without any need to break down your particles (Figure 4).

However, any A260 titration assay is heavily affected by any other UV/Vis-absorbing components in your sample. Buffer components that absorb in the UV, host cell DNA or protein will all directly skew your results – and protein or DNA contamination is common as you clean up AdV samples. By combining UV/Vis with DLS and SLS results, Stunner's measurements are more robust against contamination and matrix effects. For example, DNA contamina-

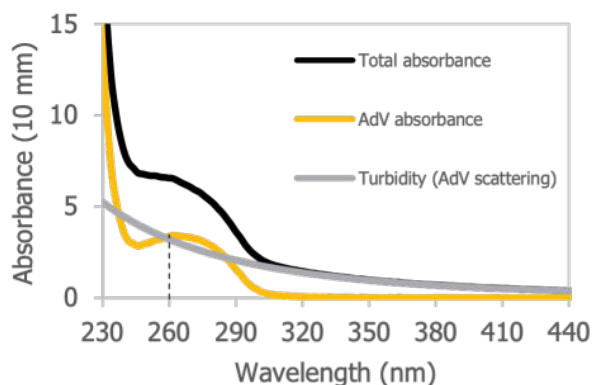


Figure 4: Stunner's turbidity correction allows for a quick and simple A260 measurement, without the hassle of SDS denaturation prep. The absorbance by the AdV (yellow) is calculated by subtracting the turbidity (gray) from the total absorbance (black). The dashed line indicates the measured A260 value.

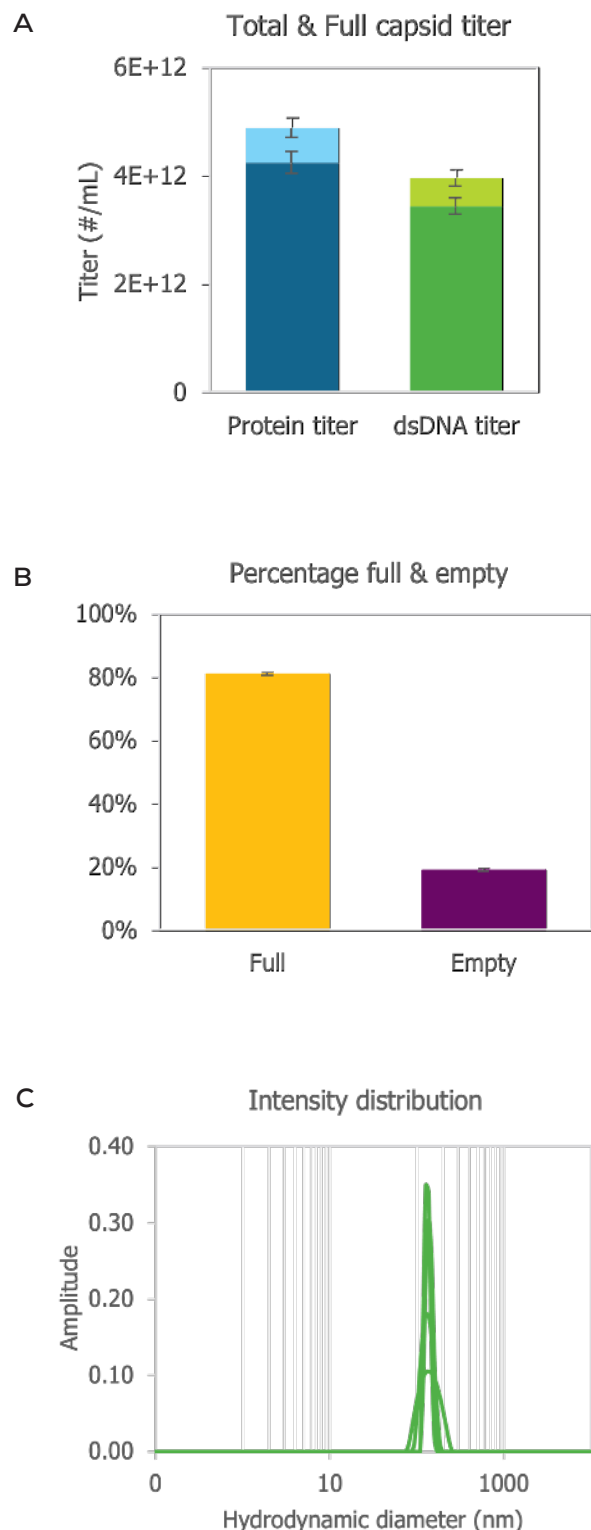


Figure 5: AdV from Vendor 1 was measured in quadruplicate on Stunner's Adeno Quant application. The number of total capsids is shown as a subset of the total protein measured (blue column), while full capsid titer is a subset of the total amount of dsDNA measured (green column) (A). Full capsid titer and total capsid titer relate to %full (yellow) and % empty (purple) data. #/mL is cp/mL for protein and vg/mL for DNA titers (B). DLS is reported as an intensity distribution (C). Error bars represent standard deviation.

tion will throw off a titer from A260, but the intensity of light scattered by AdV particles won't be impacted from excess DNA.

Know your Adeno

Stunner's Adeno Quant delivers rich, multi-attribute data for every AdV sample. After running samples, reports are immediately available on total capsid titer, full capsid titer, free & aggregated protein, free & aggregated dsDNA – and size and aggregation info from DLS is available on every AdV sample. Gathering DLS data from 2 μL of sample makes sizing information a quality attribute that can be added to any step of the process.

When checked out by Adeno Quant, the adenovirus from Vendor 1 showed high purity, titer, a high percentage of full capsids, and excellent sizing data (Figure 5). The number of total capsids averaged $4.2\text{E}12$ cp/mL with a %CV=4.9, while the full capsid titer averaged $3.4\text{E}12$ vg/mL with a %CV=4.4. Free & aggregated proteins, and free & aggregated dsDNA were about 13% of the total mass. The sample had an average % full of 81%. The size and size distribution of all four samples show excellent agreement, no aggregates, and good polydispersity.

If your sample shows monodisperse particles with a Z-average diameter between 100–200 nm, then you know runaway aggregation is no problem. Vendor 2's adenovirus sample looks like aggregation is not an issue with a Z-average diameter of 122.6 nm and a PDI <0.1 (Figure 6A, right). However, once you start to check out the whole picture Adeno Quant shows, you can spot that tons of

excess DNA is present (Figure 6A, left). The excess DNA points a finger at potential host cell DNA contamination, and would be missed by many other analytical techniques.

For Vendor 3, the balance of total protein and DNA in their adenovirus sample lined up exactly with what you'd expect from a well-purified sample (Figure 6B, left), but DLS & SLS immediately reveal the problem with this sample is a large amount of aggregation (Figure 6B, right). Stunner even synthesizes all this together by graphing all protein and DNA data as light blue and light green – indicating the protein and DNA is 'free & aggregated', and not part of any adenovirus capsid. Having all these pieces of info suggests that poor viral stability could be at fault here – since the balance of protein and DNA is plausible, but nothing is the right size for an intact adenovirus.

Conclusion

Rapid, at-line characterization of AdV is a critical piece in monitoring the manufacturing process and producing a successful batch of drug product. Current methods are cumbersome and yield highly variable results. Thankfully, Stunner's Adeno Quant snags spot-on AdV characterization quickly and easily, without any extra messy workflow, dyes, or standards. Stunner is the only tool that bridges three techniques and yields detailed results that tell a full story. With the right info, optimizing production or purification can be faster than ever, and you can build KPIs or COAs from a rapid, at-line assay ready to inform whether an AdV lot can be advanced to its next step.

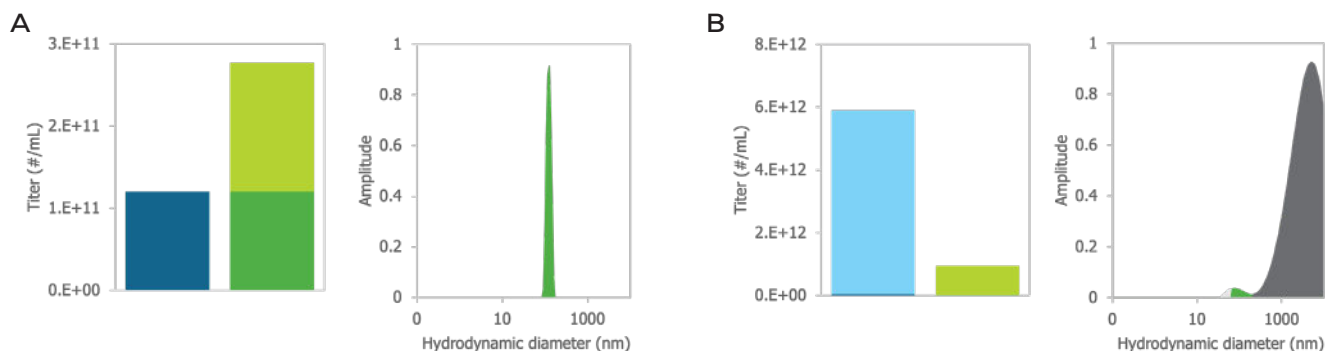


Figure 6: Adeno Quant equips you with a full picture of your sample. Vendor 2's adenovirus sample shows encouraging hydrodynamic size and monodispersity from DLS, but excess DNA (A). Vendor 3's sample has the amount of total protein and DNA you might expect, but a quick look at DLS shows massive aggregation (B). Colors represent the same info as in Figure 2.

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