

## Go even higher with Lunatic

Analyzing quantifications one by one while having to dilute highly concentrated samples is laborious and time consuming. The Lunatic platform overcomes these hurdles with a dynamic range from 0.02–200 mg/mL without ever needing to dilute.

The Lunatic is your step up to the next-generation UV/Vis reader allowing you to measure up to 96 samples in 5 minutes using only 2  $\mu$ L of an undiluted sample (Figure 1).

### Pushing the limits

A recent trend in biopharmaceuticals is to use high biologics concentrations in order to maintain efficacy when applying lower injection volumes. In order to keep up with increasing concentrations in these formulations, the Lunatic system has now been further optimized for quantification of high concentrated biologics, thereby raising its upper limit to 275 OD. This makes the Lunatic system unique in its capabilities for fast, and batch wise measurements of 2  $\mu$ L samples in a large concentration range (0.03 – 275 OD). For researchers that want to get even higher, the IgG (High concentration) application can measure highly concentrated antibodies up to 250 mg/mL.

One major component of the system allowing high OD measurements to be done with high confidence is the unique architecture of Lunatic’s microfluidic consumables (Figure 2). In addition to an input well and storage channel, these microfluidic plates and chips contain micro-cuvettes with submillimeter path lengths. As these micro-cuvettes are molded, they are fixed and accurate allowing stable and reproducible results every time. The Lunatic Plate and Lunatic Chip contain one micro-cuvette of 0.5 mm allowing measurements between 0.03 – 40 OD. The High Lunatic Plate and High Lunatic Chip however comprise two adjacent micro-cuvettes with 0.1 and 0.7 mm path lengths permitting quantification of any protein between 0.03 – 275 OD. Although these highly-concentrated protein solutions have

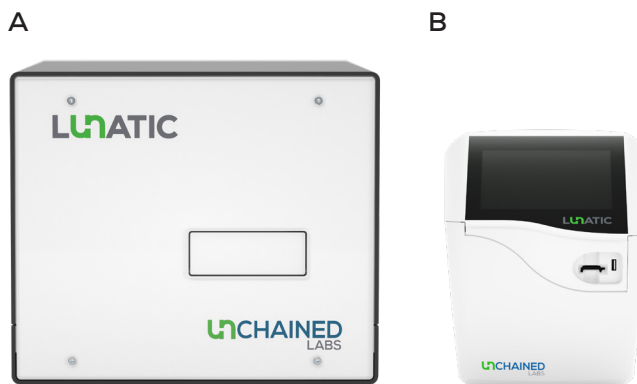


Figure 1: Lunatic: A plate reader analyzing 96 samples in 5 minutes, fit for manual or robotic sample processing (A). Little Lunatic: A chip format reader analyzing 16 samples in 2 minutes (B).

the tendency to be viscous, the microfluidic channels in the Lunatic disposables are able to capture and measure such samples with a viscosity up to 40 cP (centipoise).

The newly defined upper OD limit was validated measuring an IgG dilution series ranging from 1 up to 200 mg/mL (Figure 3). Measurements were performed on seven Little Lunatic and two of the larger Lunatic instruments. Figure 3 shows spectra for octuplicate measurements on one Lunatic (Figure 3A) and further plots the measured OD values in function of the target concentrations (Figure 3B). The table in figure 3B denotes the average %CV for all instruments assessed. The average %CV ranges from 0.5% up to 2.7% pointing out the high degree of precision. As an average might obscure large inter-instrument dispersions, instrument-specific %CV were analyzed. This intra-instrument analysis shows only a marginal extension of the average %CV range, i.e., from 0.1%–3.5%. Altogether, these results ensure the degree of precision both on the inter- and intra-instrument level.

In addition to the precision, we also examined accuracy of the results obtained. The percentage of bias looks at how closely concentrations approached the target concentrations determined

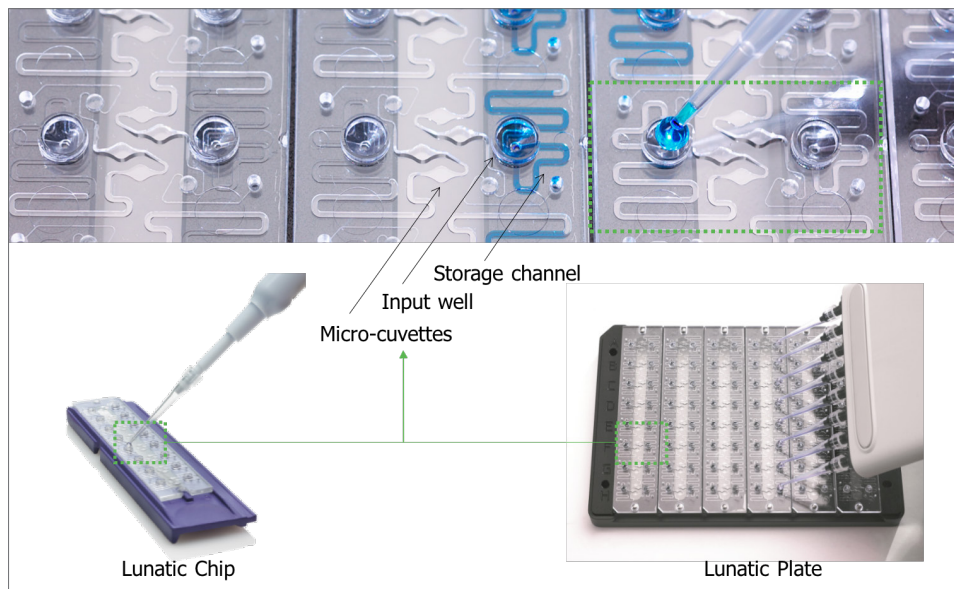


Figure 2: Different Lunatic microfluidic consumables. The High Lunatic Plate or High Lunatic Chip: includes two adjacent micro-cuvettes for a dual path length measurement (0.1 and 0.7 mm), generating a large OD measurement range (0.03 – 275 OD, 10 mm equivalent), thereby eliminating the need for sample dilution.

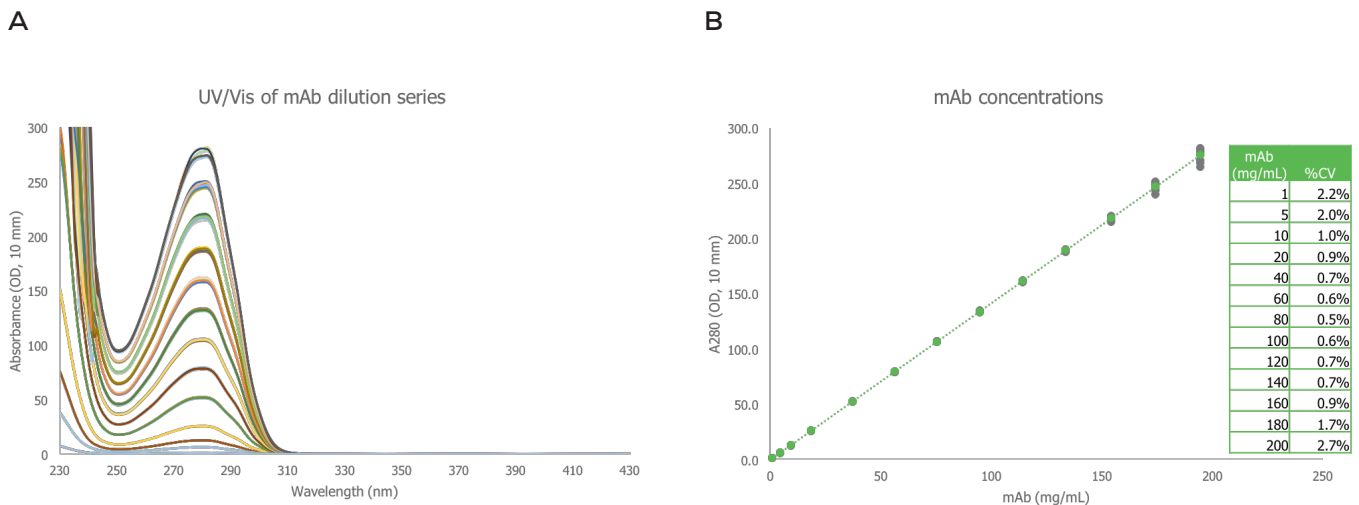


Figure 3: Results for a gravimetric IgG dilution series on seven Little Lunatic and two of the larger Lunatic instruments. Spectral results for octuplicate readouts per concentration on one Lunatic instrument (A). Plotting the average measured OD values per instrument in function of the corresponding concentrations. The green line denotes the overall average OD values (B).

on a classic 10 mm cuvette reader. The average percentage of bias for all instruments investigated ranged from -1.7% up to 3.5%, indicative for the generation of not only very precise but also very accurate results.

## Conclusion

In order to be able to keep up with current evolutions in the biopharmaceutical landscape, the Lunatic platform was further optimized to extend its dynamic range. This optimization in combination with the small, stable path lengths of the Lunatic Plates has led to an enlargement of the dynamic

range to 0.03-275 OD retaining outstanding precision and accuracy. This allows any protein sample to be measured in a high-throughput, dilution-free and very reproducible way, independent of instrument. To raise the bar even higher, a specific app for highly-concentrated IgG formulations was developed allowing quantifications of full-length IgG molecules up to 250 mg/mL.



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