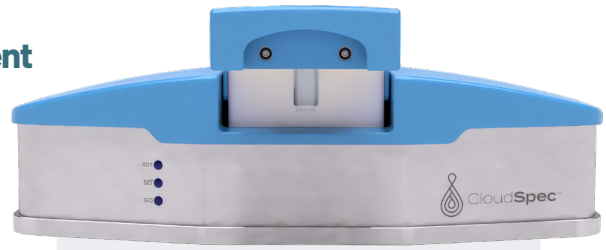
MARAMALABS

Quantification of Antibody-Conjugated RNA LNPs

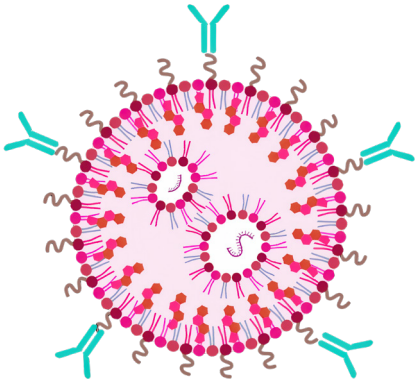
Robust UV/VIS technology | **Quantify RNA & Antibody at the same time** | **15 second measurement**

Quantify RNA and AB in a single 15 sec measurement

Scatter-free absorption (SFA) spectroscopy can accurately quantify both RNA and DNA, outperforming conventional assays such as RiboGreen[1]. This application note demonstrates that SFA can simultaneously measure RNA encapsulated within a lipid nanoparticle (LNP) and a targeting antibody (AB).



CloudSpec Nanonparticle Analyser
NO DYES OR PARTICLE DISPERSION NEEDED



Antibody coatings can enable targeted RNA or DNA delivery..

Analysing antibody-conjugated RNA-LNPs

RNA-loaded lipid nanoparticles (RNA-LNPs) deliver therapeutic RNA into cells to stimulate immune responses or treat disease. However, they tend to accumulate in the liver - often an off-target site. To reach specific cell types, such as T-cells in cancer immunotherapy, LNPs can be functionalised with targeting ligands, such as antibodies. Traditional fluorescence assays (e.g. RiboGreen for RNA, BCA for antibodies) can quantify these components, but they require separate workflows. The BCA (bicinchoninic acid) assay is prone to interference from lipids in the formulation[2]. As a result, quantifying both components in a formulation is labour-intensiveand error-prone.

Using Scatter-Free Absorbance instead

Nanoparticles inherently scatter light, which limits the use of conventional/nanovolume UV/Vis. CloudSpec's scatter-free absorption (SFA) technology eliminates this problem by removing the scattering signal entirely[3], producing a true absorption spectrum (as if the particles were transparent). Just like traditional UV/Vis, SFA obeys Beer-Lambert law, enabling precise quantification of analytes within nanoparticle formulations without interference from scattering.

CloudSpec spectra of RNA-AB-LNP

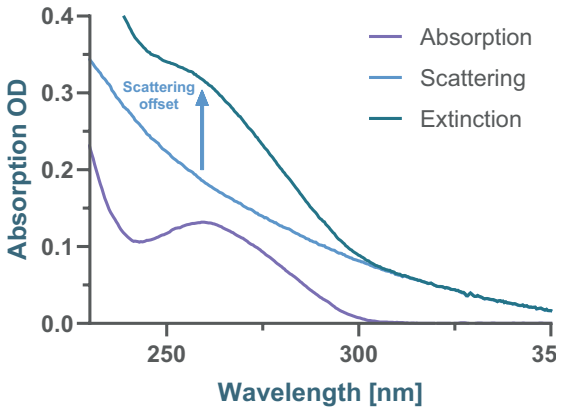


Fig 1 - CloudSpec measures a traditional UV/Vis spectrum, a scatter-free spectrum and the underlying scatter spectrum in a single 15 second measurement.

Advantage of SFA for mRNA and antibody quantification

Once a scatter-free absorption (SFA) spectrum is captured, it allows accurate quantification of multiple components within a sample, provided each has a unique and distinguishable absorption profile.

In Figure 1, traditional UV/Vis (extinction) spectra of LNPs are heavily influenced by light scattering - especially at shorter wavelengths- which can obscure true absorption features. In contrast, CloudSpec's scatter-free absorption (SFA) spectrum eliminates scattering by the design of the measurement system, not via post-processing. This is particularly valuable for quantifying weakly absorbing species like antibodies, where even small errors in the baseline would cause significant inaccuracies. This application note demonstrates that SFA's clean, scatter-free measurement enables reliable antibody quantification.

Quantifying RNA and antibody spectroscopically

Figure 2 compares the scatter-free absorption spectra of an mRNA and an antibody molecule, both measured at the same mass concentration and in the same volume. The RNA spectrum is shown in green, while the antibody spectrum is in orange. Importantly for spectroscopic analysis, the antibody absorbs about 25 times less light than RNA for the same mass.

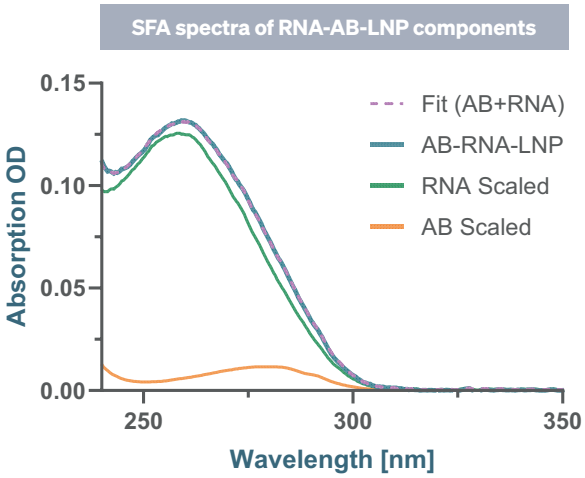


Fig 2. SFA spectra of AB-RNA-LNP and each component.

In SFA, as in standard UV/Vis spectroscopy, the total absorption measured is the sum of each components, scaled by their concentration and extinction coefficient (magenta Fit dashed line).

Even though antibodies contribute a small signal relatively, the combined SFA spectrum (blue line) still enables accurate concentration measurement of both mRNA and antibody. By using spectral deconvolution - a method of mathematically separating overlapping signals - and the component spectra, CloudSpec's software can determine the concentration of both mRNA and antibody in a single measurement.

Helpfully, the individual component spectra need to be measured once only and can then be used for the analysis of similar samples.

Quantification of RNA and antibody in intact LNPs

In the example below*, mRNA and antibody concentrations in an mRNA-LNP sample spiked with AB were quantified using individual spectra of the components[4]. The objective was to demonstrate the SFA's ability to accurately, selectively, and linearly quantify both payloads within a single 15-second scan. To assess sensitivity and selectivity, one analyte was systematically varied while the other was held constant. Measured concentrations were then plotted against known input values to evaluate the assay linearity.

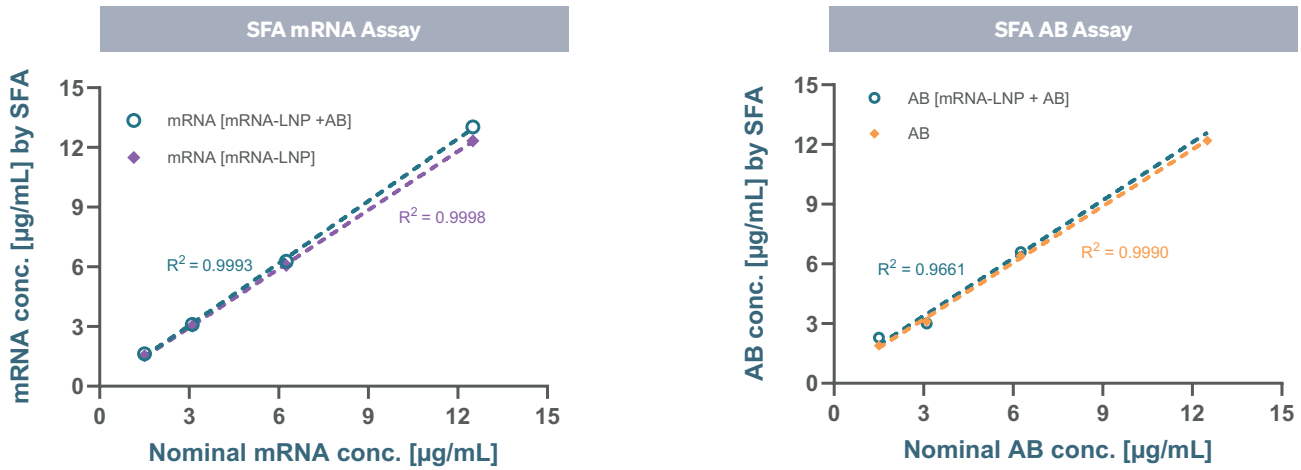


Fig 3 (a) RNA concentration (left) and (b) antibody concentration (right) against the expected concentration. Each plot shows the free analyte measurement and as a component of the RNA-LNP + AB mixture. The linear trends highlight the excellent selectivity, specificity, and measurement accuracy in both scenarios.

Summary

SFA enables simultaneous quantification of multiple analytes in LNP formulations, requiring only that their spectral signatures are distinct. The technique is both selective and specific, delivering accurate readings for each component in a single scan. It serves as a powerful alternative to traditional assays by streamlining workflows and eliminating the need for separate tests or complicated sample preparation.

*Data supplied by Johanna Simon of Merck Life Science KGaA, Darmstadt, Germany. Email johanna.simon@emdgroup.com

[1] Le Ru, et al, Nano Lett. 2025, 25, 16, 6813–6819
[2] Kessler, et al, Anal Biochem, 1986, 159(1), 138-142
[3] https://maramalabs.com/applications
[4] https://youtu.be/DD9aYSy9JuY, speaker Johanna Simon, EMD Group