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Measuring Intact (Full-length, Capped, Tailed) mRNA in a Single Assay: 5′CapQ

InDevR partners with Aldevron to describe how the VaxArray 5′CapQ assay can be used to measure intact mRNA in a single assay to streamline bioprocess development

By Erica Dawson, PhD, and Rebecca Young, PhD

In the years after the 2020 COVID-19 pandemic, it is clear that mRNA as a modality for vaccines and therapeutics is here to stay based on the number of mRNA programs currently in clinical development. The speed at which safe, effective COVID-19 vaccines were developed and licensed required Herculean efforts—and, out of necessity, required a reliance on existing analytical methodologies quickly adapted to the task at hand for assessing these life-saving vaccines.

InDevR's early conversations with mRNA vaccine manufacturers made it apparent that bioprocess optimization of mRNA vaccine constructs was often slowed by analytical methods reliant on expensive, complex instrumentation. These methods are often performed at a centralized analytical lab or CDMO by a scientist with specialized training, and with sample analysis occurring only after a substantial time in a queue.

When more sophisticated methods are not accessible for reasons of cost or complexity, reliance on sub-optimal techniques such as semi-quantitative dot blot employing an anti-m7G antibody may also be utilized. Recently highlighted methods to assess analytics such as mRNA integrity, 5′ capping efficiency, and polyA tailing detailed in recent guidance documents[1] certainly have their place and can provide rich mRNA characterization needed at certain phases of a program's development. However, it seemed there was room in the toolbox for rapid, easy to use, at-line methods that can be utilized as screening tools to inform the bioprocess more immediately.

VaxArray: a foundation for vaccine analytics

InDevR has offered the VaxArray platform of microarray-based, multiplexed analytical solutions to the vaccine industry since 2014. The VaxArray platform consists of a benchtop fluorescence-based imaging system, 21 CFR part 11-compatible software, and a suite of off-the-shelf and custom reagent kits, as shown in *Figure 1*. Applications currently utilizing VaxArray analytics include bioprocess development and optimization, QC release testing, and clinical studies.

While historically focused on traditional protein-based vaccine analytics, InDevR, in response to market needs for mRNA analytical tools in recent years, has been expanding a suite of VaxArray microarray-based analytics offerings to include detection and characterization assays for mRNA and other nucleic acids that offer high ease of use and rapid times to result for high impact at the bioprocess bench and beyond.

Measuring intact mRNA: 5′CapQ assay approach

As shown in the highlighted schematic at left in *Figure 1*, the 5′CapQ assay utilizes an anti-5′ cap antibody printed in replicate

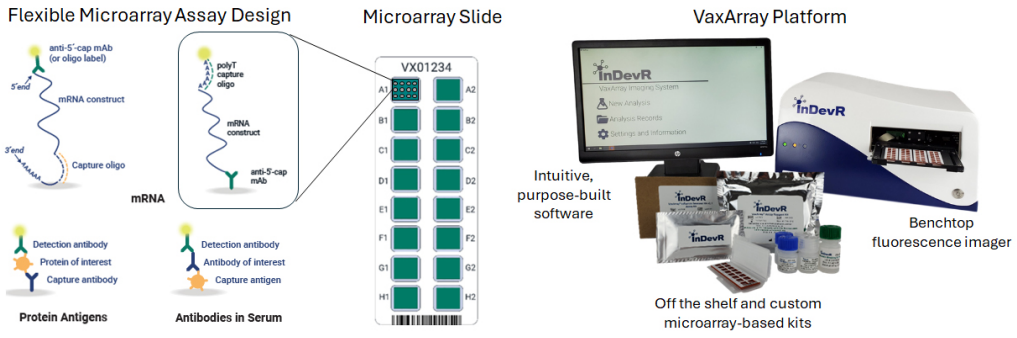

Figure 1. (*Left*) examples of flexible VaxArray microarray-based assay designs, with 5′CapQ assay schematic highlighted, (*Center*) a schematic of a single VaxArray microarray slide with 16 replicate arrays, (*Right*) components of the VaxArray Platform.

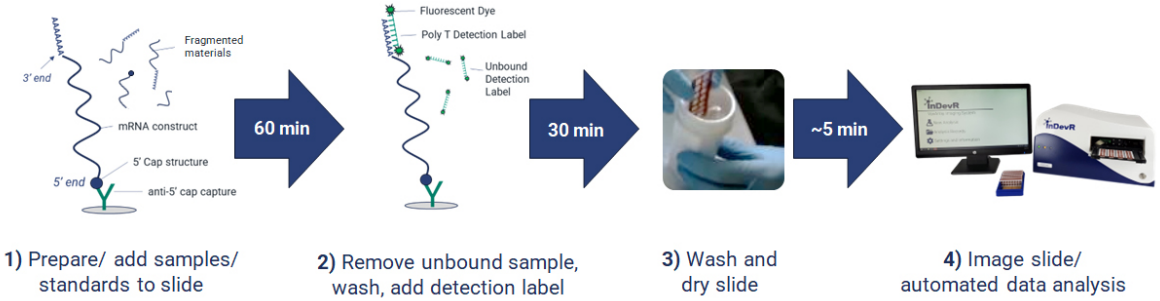

Figure 2. 5′CapQ assay procedure, with a time to result of < 2 hours including sample preparation.

spots on the microarray substrate to capture the 5′ cap of an mRNA construct of interest (confirmed reactivity to Cap 0 and Cap 1 structures, including caps added with popular commercial 5′ capping kits). The mRNA is subsequently labeled with a fluorescent polyT oligonucleotide that binds to the 3′ polyA tail. This detection scheme ensures that mRNA that is cap-less, tail-less, or fragmented will not be detected, enabling a measurement of only the fully intact mRNA in a single assay. The kit is provided with all associated ancillary reagents needed for mRNA incubation and labeling steps. The 5′Cap***Q*** assay procedure is outlined in *Figure 2*.

A unique measurement of intact mRNA

Currently, manufacturers make up to three separate measurements for mRNA integrity, 5′ capping efficiency, and 3′ poly(A) tailing to estimate how much mRNA is intact from cap to tail. mRNA integrity is typically measured via an electrophoretic method, and 5′ capping efficiency and 3′ poly(A) tailing are increasingly measured via LC and LC-MS based methods and typically require an upfront enzymatic digestion or cleavage step prior to analysis, which eliminates the tie between the full-length mRNA sequence and the cap or tail.

In contrast, the 5′Cap***Q*** assay provides a single measurement of the total amount of intact mRNA in a sample that is both capped and tailed. This can be a relative measurement if comparing different samples of the same construct (say as a function of differing IVT conditions, enzymatic capping conditions, or purification steps), or when compared to a standard of known intactness.

Alternatively, a *quantitative* assessment of intact and capped mRNA can be determined if a user-provided, sequence matched standard is analyzed as a calibration curve alongside the unknown samples of interest. If the standard has been measured for integrity and 5′ capping efficiency via alternative methods, these values can be utilized to assign a known "capped, intact" value to the standard.

To generate the data in *Figure 3* and *Table 1*, we mixed intact (full-length, capped) and uncapped versions of the same mRNA construct (commercially available construct coding for GFP) in different ratios to create a series of samples tha t should differ by 5% intactness. When assessed using the 5′Cap***Q*** assay, the expected step function is observed as the amount of intact, capped mRNA decreases. The standard used for quantification was the intact, capped material (which had known integrity/capping efficiency). As shown in *Table 1*, the accuracy of the measurement was quite high, with all values producing intactness values close to expected.

To highlight the utility of the assay, Aldevron provided InDevR with four samples of the same underlying mRNA construct and same polyA tail length generated under different enzymatic 5′ capping conditions. Aldevron assessed the samples via semi-quantitative m7G dot blot assay for comparison, and intactness/integrity measurements were made via capillary electrophoresis. As shown in *Table 2*, all four samples had similar intactness, but differed in 5′ capping efficiencies by dot blot analysis.

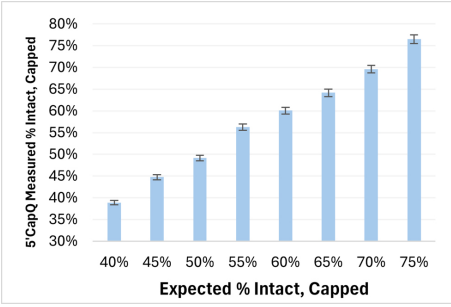

Table 1.

Expected Value	Measured % Intact, Capped (95% CI)	Difference from Expected
40%	38.9 (38.4 – 39.4)	-1.1%
45%	44.8 (44.1 – 45.3)	-0.2%
50%	49.2 (48.5 – 49.8)	-0.8%
55%	56.3 (55.5 – 57.0)	+1.3%
60%	60.1 (59.3 – 60.8)	+0.1%
65%	64.2 (63.3 – 65.0)	-0.8%
70%	69.6 (68.7 - 70.5)	-0.4%
75%	76.5 (75.5 - 77.5)	+1.5%

Figure 3 and Table 1. 5′CapQ measured % intact, capped mRNA in samples prepared by mixing intact and uncapped, sequence-matched mRNAs and measuring against the intact material (% intact known). Error bars in figure at left are upper and lower 95% confidence intervals (95%CI), with accuracy of the measurement also shown in the table at right.

caption

Table 2. 5′Cap***Q*** assay analysis of Aldevron materials along with associated % intact mRNA and capping metrics

Sample	% Intact mRNA (provided by Aldevron)	Relative 5' capping efficiency by m7G dot blot (provided by Aldevron)	% Intact, Capped mRNA (5' Cap***Q*** Assay)
Sample 1	77.0%	High	78.0%
Sample 2	82.9%	Moderate	54.9%
Sample 4	84.1%	Moderate	40.8%
Sample 3	83.9%	Low	Unreactive
Standard	73.7%	90%	N/A

A standard for quantification was also provided (same mRNA construct sequence and polyA tail length) with known integrity and high 5′ capping efficiency (> 90%). The 5′Cap***Q*** assay was conducted by analyzing a dilution series of the standard (assuming 90% capping efficiency and 73.7% integrity based on Aldevron's measurements) alongside the unknown samples.

The % of intact, capped mRNA of the unknown samples was then back calculated from the standard curve. Given that the 5′Cap***Q*** assay only measures mRNA that is intact and has both a cap and a tail, we would expect the 5′Cap***Q*** assay result to be an approximate convolution of the intactness and 5′ capping measurements (assuming similar tailing efficiencies). Given this, the four samples showed the expected 5′Cap***Q*** assay trend in that the sample with the highest capping showed the highest 5′Cap***Q*** result, and the two samples only moderately capped produced an intermediate 5′Cap***Q*** assay result. Sample 3 was expected to have low capping efficiency by dot blot and was below the detection limit of the 5′Cap***Q*** assay.

The VaxArray approach provides a high-throughput assessment of a variety of bioprocess conditions, simultaneously assessing capping efficiency and intactness. This allows for fast turnaround times and increased throughput for development of new bioprocess conditions and for assessment of RNA CQAs. Minimizing the time and complexity of these analyses is a great support to programs which often rely on quick results and require reliable data.

InDevR's 5′Cap***Q*** assay for the VaxArray Platform is a rapid, 2-hour solution for measuring intact mRNA molecules from cap to tail at the bioprocess bench and can be successfully executed with no specialized training other than basic laboratory and pipetting skills. 5′Cap***Q*** can offer relative quantification without a standard as a function of in vitro transcription (IVT) or enzymatic capping optimization to maximize yield of intact mRNA or can offer absolute quantification if assessed alongside a user-provided, matched standard of known intactness and capping efficiency. We hope the availability of new tools for mRNA analytics will help expand the toolbox to enable more rapid development and optimization of mRNA vaccines and therapeutics. GEN

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Reference

1. Analytical Procedures for mRNA Vaccine Quality (Draft Guidelines)—3rd Edition. USP-NF, (accessed 2025 Jan 13).