Simplifying Progress

Effect of Mg^{2+} Ion Concentration on IVT Reaction Kinetics Determined by Novel Rapid Analytical HPLC Assay

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Analytical Bottleneck in IVT Optimisation

The IVT reaction is one of the most expensive steps in mRNA production process and its optimization to reach high mRNA yield is of key importance. Standard mRNA quantification techniques like absorbance and fluorescence based assays are time consuming and cannot be performed at line as the IVT reaction progresses. In addition, other reaction components like nucleotides and pDNA interfere in the analytical results and reduce the method's accuracy. A new approach shown here uses CIMac PrimaS® analytical HPLC column to separate and quantify several key IVT components with a very short run time, enabling fast "at line" tracking.

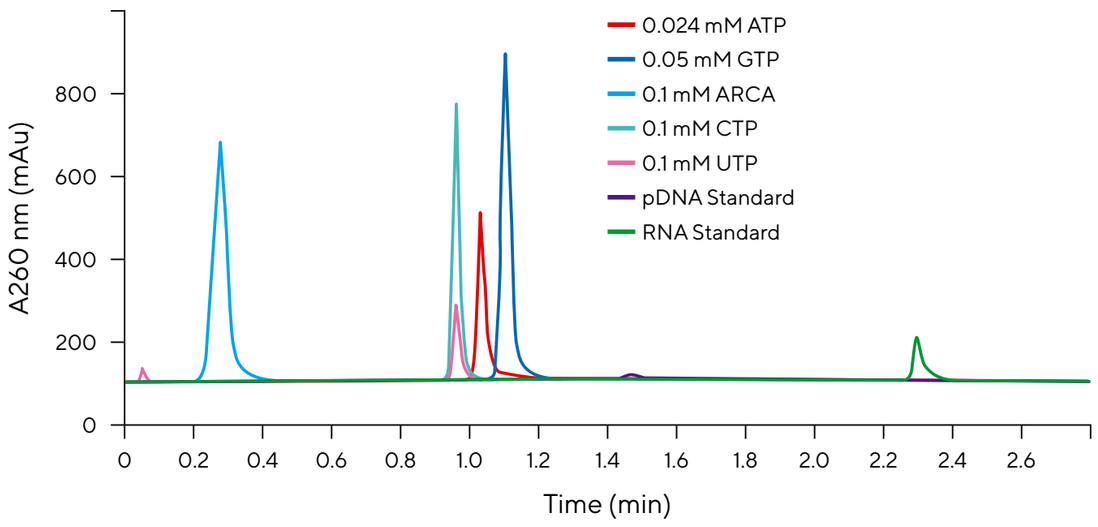

Figure 1: *Overlay of IVT Mixture Components on CIMac PrimaS®*
Buffers: MPA (50 mM HEPES pH 7), MPB (50 mM HEPES, 200 mM sodium pyrophosphate, pH 8.5). Method: 0–1 min (100% MPA), 1–1.8 min (gradient to 20% MPB), 1.8–2.5 min (gradient to 80% MPB). Full method not shown. PATfix® HPLC system, UV absorbance at 280 nm, flow rate 2 mL/min, injection volume 25 µL.

The HPLC method provides information on mRNA yield, consumption of nucleotides, and capping reagents in a single 8 minute assay. The column separates nucleotides (CTP/UTP, ATP, GTP), capping reagent ARCA , mRNA and pDNA. Based on peak areas, standard curves for concentration determination can be prepared as shown in Figure 2.

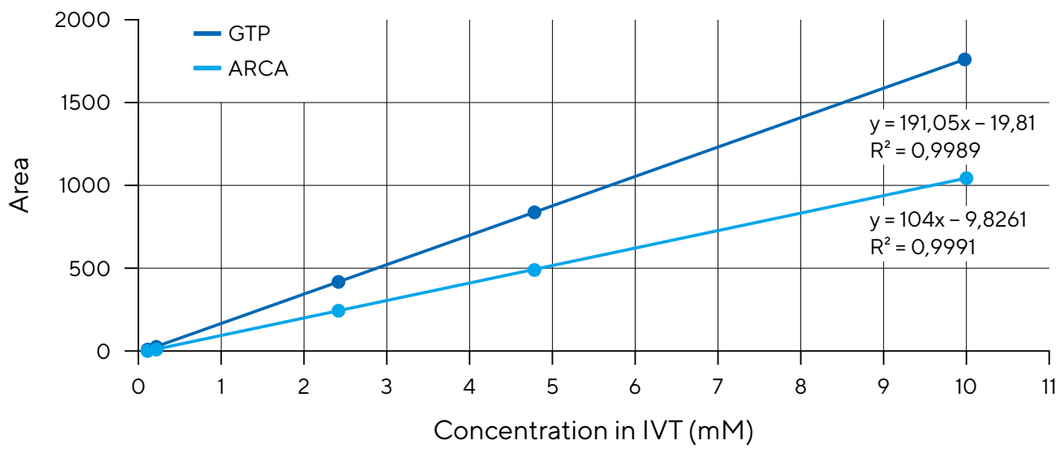

Figure 2: *Standard Curves for ARCA and GTP Peak Areas.*
Plot showing Area vs concentration of ARCA and GTP in IVT (200 times diluted; injected 25 µL). The method is linear in the selected range. Other calibration curves not shown here.

Optimization of $MgCl_2$ Concentration in IVT Using PrimaS® Analytics

The concentration of $MgCl_2$ is one of the critical parameters in the IVT reaction. Seven identical reactions (20 µg/mL linear pDNA, 500 U RNA polymerase per µg pDNA, 4 mM ATP, CTP, UTP and GTP each, 1U/µL RNAse inhibitor, 1U/mL pyrophosphatase), varying only in the concentration of $MgCl_2$ (6 mM, 9 mM, 12 mM, 15 mM, 20 mM, 25 mM, and 50 mM) were analysed at different time points (0 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, and 4 h) on CIMac PrimaS® (N = 1 for each reaction). An overlay of chromatograms at all time points in Figure 3 shows consumption of nucleotides, and production of mRNA. Depletion of nucleotides can be observed after 1 h.

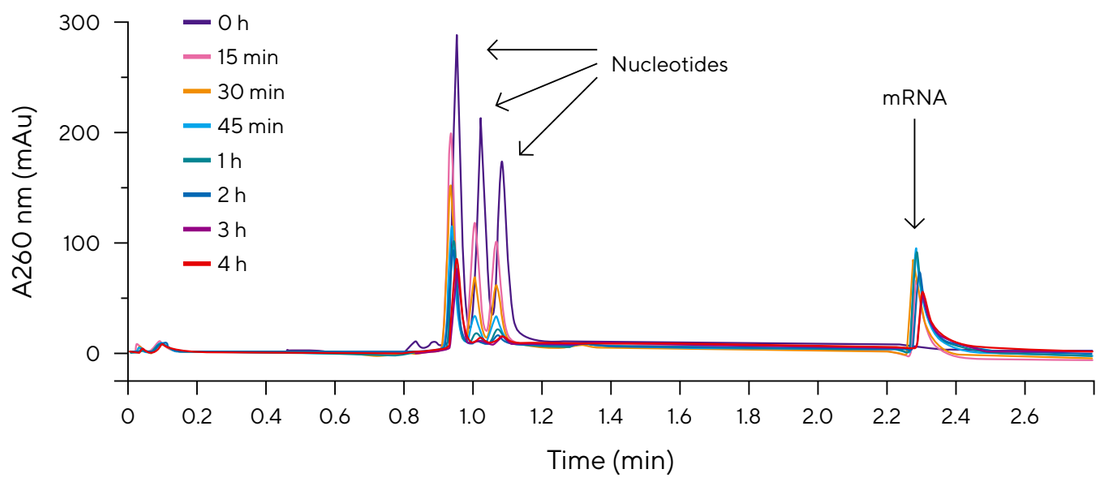

Figure 3: *Time Point Analysis of Nucleotide and mRNA Content During IVT Progression.*
Sample: IVT sample, inactivated with EDTA a nd diluted in MPA (200 fold dilution).

Kinetics of the IVT reaction are strongly dependent on concentration of magnesium in the reaction (Figure 4). Most conditions result in a plateau after 1 h incubation, possibly due to depletion of nucleotides, and intermediate concentrations of $MgCl_2$ (12, 15, 20 mM) show higher mRNA yield at this time-point. Extension of the reaction beyond 1 h results in further increase of mRNA yield at extreme conditions (6 mM, 50 mM).

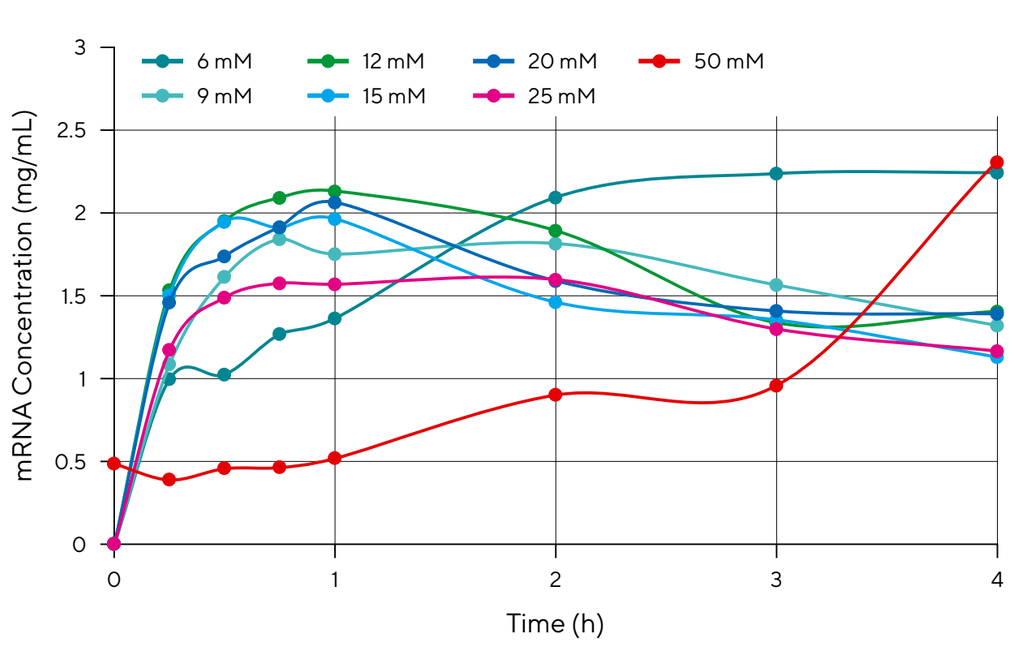

Figure 4: *Kinetics of the IVT Reaction at Different Concentrations of $MgCl_2$.*
Samples: IVT sample, inactivated with EDTA and diluted in MPA (200-fold dilution).

Conclusion

- A new analytical assay with CIMac PrimaS® shows different selectivity for all of the IVT mixture components, requires minimal sample preparation, and provides rapid quantitative results, "at line."
- The method allows measurement of reaction kinetics and productivity in different conditions.
- Depletion of reagents (capping reagent, nucleotides) can be detected.
- The same method allows in process control during mRNA purification.
- The method was applied here to investigate the effect of magnesium ion concentration on mRNA production kinetics.