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Application Note

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Purification of mRNA by Affinity Chromatography on CIMmultus® Oligo dT Column

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Abstract

Increasing demand for messenger RNA (mRNA) as therapeutic product requires efficient and scalable purification methods. Research and laboratory scale purification often employs a variety of precipitation approaches. While suitable for small scale production, chromatographic methods are often preferred in large scale manufacturing. Chromatography can provide better scalability, improve automation, and reduce handling of the drug substance.

Different modes of chromatography can be employed for purification of nucleic acids, including ion exchange, hydrophobic, reversed phase, and affinity chromatography. The latter exploits the hybridisation affinity between the poly-A tail on the mRNA and a poly-dT chain coupled to a chromatographic stationary phase. An affinity approach allows rapid implementation with minimal optimisation steps.

Here, CIMmultus® Oligo dT, a monolithic stationary phase is used for purification of mRNA from a capping mix of Luc2 RNA. The large channels and convective mass transfer within the stationary phase eliminate shear forces and allow high flow rates, which leads to very short purification times and high recoveries.

Introduction

The increasing demand for messenger RNA (mRNA) as therapeutic product requires larger production scales, and in turn more efficient extraction techniques. mRNA can be produced by in vitro transcription reactions (IVT) or isolated from eukaryotic cells. One of the most convenient techniques for its extraction is the use of oligo deoxythymine (dT) coupled to a solid support. Oligo dT hybridises to the poly-adenylated tail which is present on most eukaryotic mRNAs, or synthetised onto the molecule during IVT. Contaminant impurities, such as proteins, unreacted nucleotides, plasmid DNA, CAP analogues etc, lack the poly-A moiety and are not retained on the solid support.

CIMmultus® Oligo dT is a chromatography column with Oligo dT ligands covalently bound on its surface. The sample containing poly-adenylated mRNA is loaded onto the column in a high salt concentration buffer. Salt ions screen the electrostatic repulsion between the negatively charged backbones and allow interaction between the Oligo dT and poly-adenylated tail of mRNA. Prior to elution, a wash step at reduced salt concentration removes unspecifically bound contaminants. Elution of mRNA occurs under mild conditions in low conductivity buffer at neutral pH. In the absence of salt, electrostatic repulsion between the negatively charged backbones of Oligo dT and poly-adenine destabilises the T–A pairs and releases mRNA from the column.

Materials

BIA Separations PATfix® HPLC System or equivalent; CIMmultus® Oligo-dT, 1 mL; IVT mix (Luc2 SNIM RNA Capping mix, 0.5 mg/mL); Binding buffer: 50 mM Na-phosphate, 2 mM EDTA, 250 mM NaCl, ph 7.0; Washing buffer: 50 mM Na-phosphate, 2 mM EDTA, pH 7.0; Elution buffer: 10 mM Tris, pH 7.0; Loop volume: 1 mL; Load volume: 0.5 mL.

Methods

Equilibrate the column by flushing with at least 10 column volumes (CV) of binding buffer. Load sample and wash the loop with 8 CV binding buffer, wash the column with 4 CV of washing mobile phase, elute mRNA from the column with 8 CV elution buffer.

Results

CIMmultus® Oligo dT was loaded with IVT reaction mixture containing approximately 180 µg of mRNA.

The chromatographic profile (Figure 1) shows a large flow through fraction which is expected to contain nucleotides, substrates involved in capping reaction and mRNA lacking poly-A tail. These predominantly should not interact with the column in high salt buffer. Bioanalyzer results (Agilent 2100 Bioanalyzer using RNA 6000 Nano kit) of the load, flow through and elution fractions show that full size mRNA containing poly-A tail is retained on the column and can be recovered with elution buffer (Figure 2). The concentration of mRNA in elution fraction was determined spectrophotometrically with the NanoDrop One (Thermo Scientific). The recovery of the purification process was calculated to be 80%. An intermediate wash step before elution lowers the salt concentration in the elution fraction, eliminating the need for a desalting step.

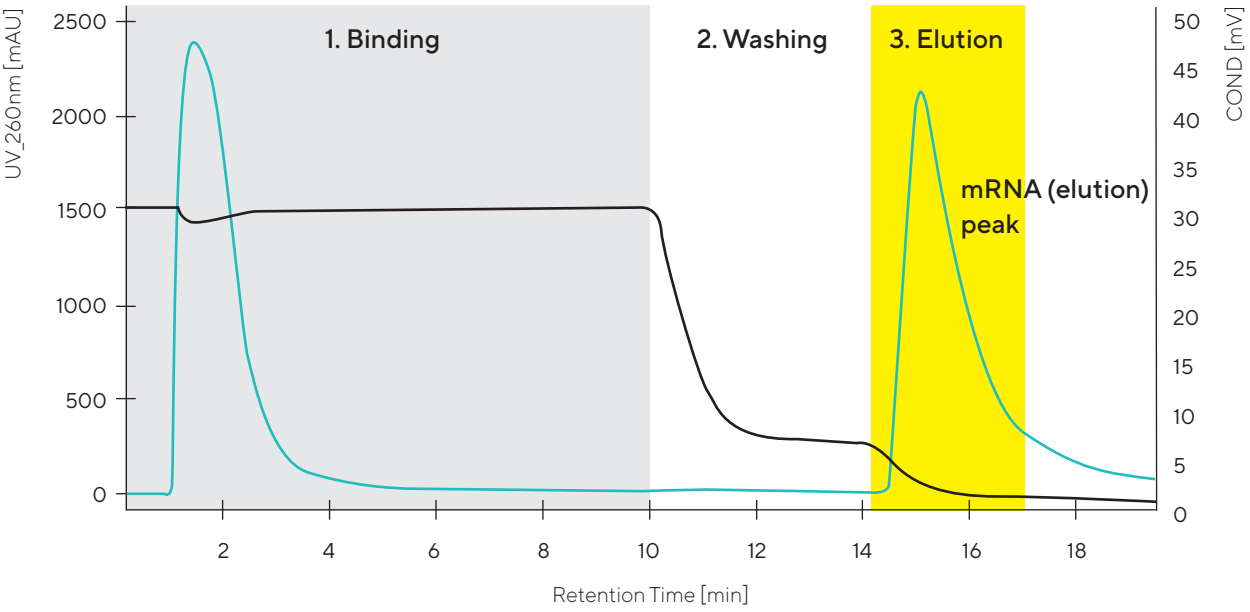

Figure 1: Chromatographic profile of IVT mix loaded to oligo dT column.
Black line represents conductivity, whereas red line indicates the UV absorbance at 260 nm.

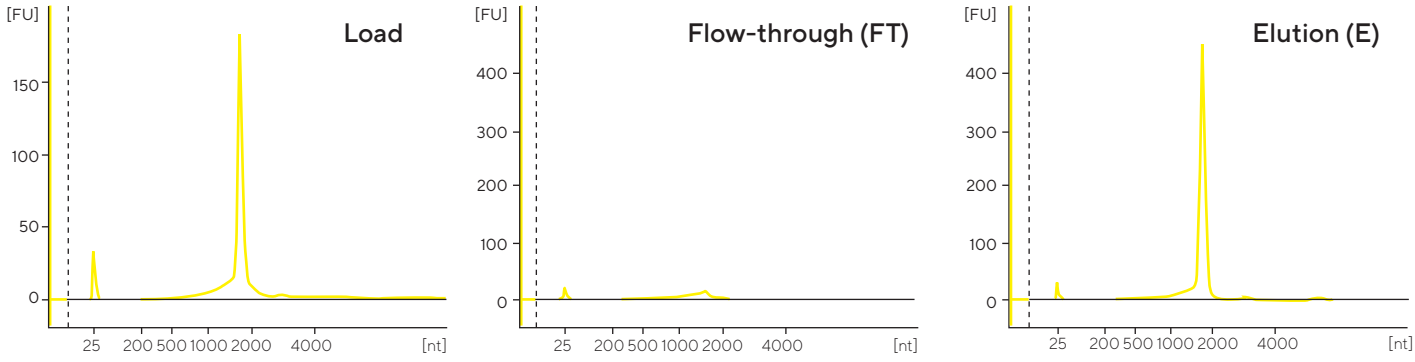

Figure 2: Electropherograms show CGE profiles of the starting material (load to the column) and collected flowthrough and elution fraction.

Conclusion

Chromatography can be used to rapidly isolate and purify mRNA containing a poly A tail. CIMmultus® Oligo dT can selectively remove impurities from the sample and is a convenient approach for initial purification of mRNA. With high flow rates and low shear forces, monoliths offer an efficient and mild approach to purify labile mRNA molecules. A polishing chromatography step would be recommended depending on the purity requirements.

References

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