AN062 Purification of messenger RNA by affinity chromatography on CIMmultus™ Oligo dT column

The increasing demand for messenger RNA (mRNA) as therapeutic product requires larger production scales, and in turn more efficient extraction techniques. Messenger RNA 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, partial transcripts, dsRNA side products and enzymes lack the poly-A moiety and are not retained on the solid support.

Chromatography using a solid phase consisting of large channels, such as monoliths, allows high flow rates and low shear forces. This can have a positive impact on recovery and productivity in purification of biologics. In addition, chromatography offers a closed system to minimise the risk of cross-contamination or exposure to RNase degradation, and an easily scalable platform.

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. Before product elution, a wash step at reduced salt concentration removes unspecifically bound contaminants. Elution of messenger RNA 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.

Chromatographic conditions:

HPLC system:	BIA Separations PATfix™ HPLC System or equivalent
Monolithic column:	CIMmultus™ Oligo-dT, 1 mL
Mobile phases:	Binding: 50 mM Na-phosphate, 2 mM EDTA, 250 mM NaCl, pH 7.0 Washing: 50 mM Na-phosphate, 2 mM EDTA, pH 7.0 Elution: 10 mM Tris, pH 7.0
Flow rate:	1 mL/min
Sample:	IVT mix (Luc2 SNIM RNA Capping mix, 0.5 mg/mL) Sample diluted with 50 mM Na-phosphate, 2 mM EDTA, 1 M NaCl, pH 7.0 in ratio 4:1
Load volume:	0.5 mL
Loop volume:	1 mL
Detection:	UV absorbance at 260 nm

Method:	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.

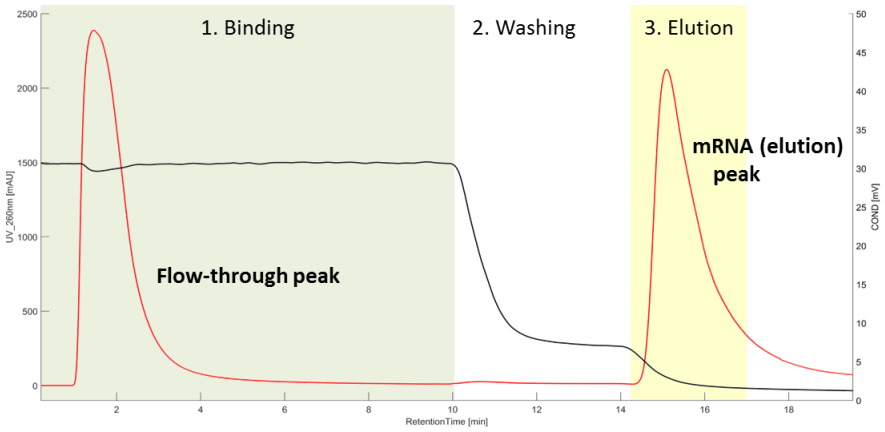

Figure 1: Chromatographic profile of IVT mix loaded to oligo dT column. Black line represents conductivity, whereas red line indicates the UV absorbance at 260 nm.

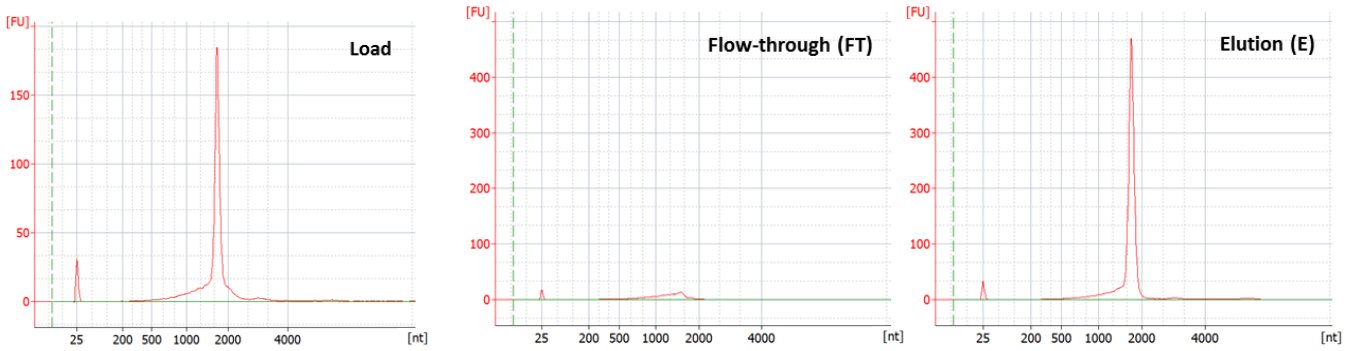

Figure 2: Electropherograms show CGE profiles of the starting material (load to the column) and collected flow-through and elution fraction.

Conclusions

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

- Aviv, Haim, and Philip Leder. "Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose." Proceedings of the National Academy of Sciences 69.6 (1972): 1408-1412.
- Sambrook, Fritsch and Maniatis, Molecular cloning: a laboratory manual, 3rd edition (2001)

Ordering information

Catalog No.	Product description
311.1218-2	CIMmultus™ Oligo dT18-1 Advanced Composite Column (Pores 2 µm)
411.1218-2	CIMmultus™ Oligo dT18-8 Advanced Composite Column (Pores 2 µm) (422.0900 adapter included)
611.1218-2	CIMmultus™ Oligo dT18-80 Advanced Composite Column (Pores 2 µm)
811.1218-2	CIMmultus™ Oligo dT18-800 Advanced Composite Column (Pores 2 µm)

Special thanks to Ethris GmbH under the framework of EU Intenso project (2012-2017) for providing IVT capping mix.

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