AN063 Preparation and testing of oligo dT column utilizing CIMmic™ platform

Pre-activated CIMmic™ monolithic columns are cost efficient tools for screening of immobilisation conditions and small scale proof-of-concept testing of custom affinity columns and enzymatic reactors. Each column is assembled from a dedicated housing and discs containing a chromatography medium. With a bed volume of 100 µL, sample requirements are minimal, while inserting multiple discs in the housing adapts the column volume to application requirements. Different surface modifications of the discs enable immobilisation of a wide variety of ligands.

The increasing demand for messenger RNA (mRNA) as a therapeutic product requires larger production scales, and in turn more efficient extraction techniques. One of the most convenient techniques for its extraction is the use of oligo deoxythymidine (dT) coupled to a solid support [1]. Oligo dT hybridises to the poly-adenylated tail which is present on most eukaryotic mRNAs, or synthetised onto the molecule during IVT, while other contaminant impurities (proteins, unreacted nucleotides, plasmid DNA, CAP analogues, partial transcripts, dsRNA side products and enzymes) lack the poly-A moiety and do not adhere to the solid support.

Amino-modified oligo dT (NH2-C6-dT18) was immobilized on one of the representatives of CIMmic family called carboxy imidazole preactivated (CDI) monolith which is also successfully used for the covalent immobilisation of proteins [1,2,3], peptides and other amine or thiol containing molecules, such as amino-modified oligo- or polynucleotides. The immobilisation was performed by following the manufacturer's protocol [4] and the functionality was confirmed by measuring the amount of bound and eluted mRNA.

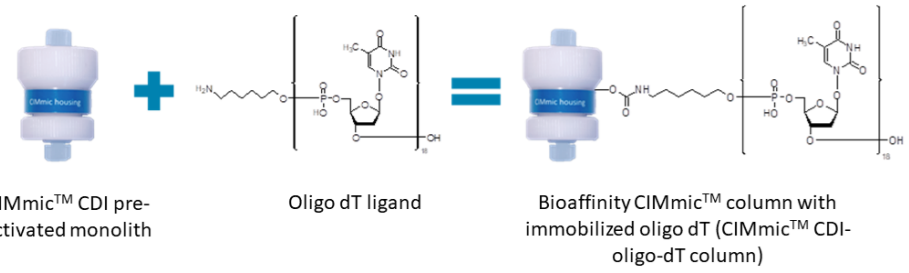

Figure 1: Schematic representation of covalent oligo dT coupling onto CIMmic CDI monolith. Carbamate bond between monolith surface and amino group of amino-modified oligo dT establishes a strong covalent immobilisation.

Chromatographic conditions:

Monolithic column:	CIMmic™ CDI-oligo-dT (oligo dT immobilized onto CIMmic CDI); bed volume 0.1 mL
Mobile phases:	Binding: 50 mM Na-phosphate, 250 mM NaCl, 2 mM EDTA, 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
Load volume:	100 µL
Detection:	UV absorbance at 260 nm
Method:	mRNA loading in binding buffer (3 min), wash (4 min), elution in step gradient (3 min)

Results

Successful coupling of the ligand does not directly result in a functional monolith, therefore a final product has to be tested for a specific application. The ease of operation together with a small size of CIMmic-based columns enable their application for fast and efficient isolation of small amounts of target molecules in micro-miligram range. For this reason, information on the quantity of processed mRNA in a single run on CIMmic™ CDI-oligo-dT was determined.

Different amounts of mRNA (Luc2 SNIM RNA Capping mix) ranging from 10 to 135 µg were injected onto CIMmic™ CDI-oligo-dT column in 50 mM Na-phosphate, 250 mM NaCl, 2 mM EDTA, pH 7.0 buffer and eluted with 10 mM Tris, pH 7.0 buffer. The amount of mRNA in unbound and bound fraction was quantified by UV-Vis measurement with NanoDrop One (Thermo Scientific).

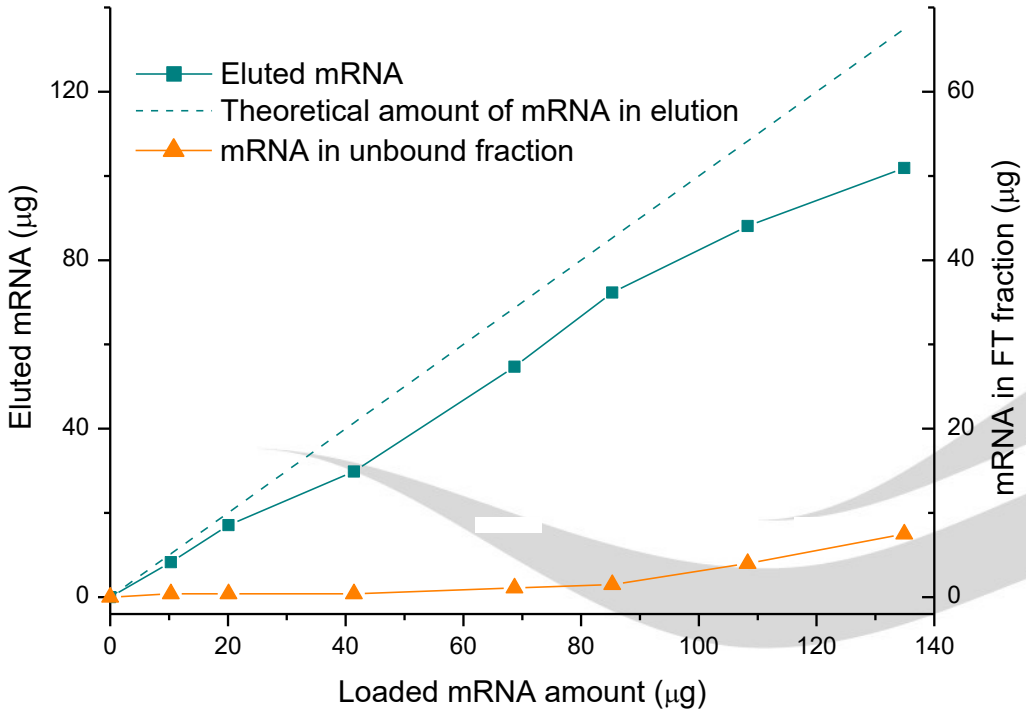

Figure 2: The amount of mRNA in FT and elution fraction. The column dynamic binding capacity for mRNA at flow rate of 1 mL/min (10 CV/min) is approximately 1 mg mRNA per mL of chromatographic support.

Figure 2 shows a linear increase of mRNA in eluted fraction with regards to the amount of loaded material up to 100 µg injection (corresponding to 1 mg mRNA per mL of support). Above this value, a negative deviation starts occurring, which correlates with a small amount of mRNA found in an unbound fraction. However, even at 135 µg loading only 6% of mRNA was unbound. It is anticipated that, at high mRNA loadings, there is an increasing shielding effect of free oligo dT ligands by previously bound mRNA, resulting in a lower binding efficiency for incoming mRNA from the sample. The isolation yield (mRNA in elution divided by total mRNA amount) at 100 µg mRNA load is 80-85%, which is an excellent result for such a delicate molecule as mRNA.

Conclusions

Carboxy imidazole (CDI) pre-activated CIMmic™ columns are a cost-efficient solution for a development of small-scale affinity chromatographic monoliths. This application note has shown the example of a successful coupling of oligo dT ligand onto CIMmic™ CDI platform. The prepared affinity column can be used for small scale isolation of up to 1ooo µg mRNA per mL of column in a single, very fast chromatographic run. Monoliths offer an efficient and mild approach to purify labile mRNA molecules, with high flow rates and low shear forces.

References

- [1] AN062: Purification of messenger RNA by affinity chromatography on CIMmultus™ Oligo dT column
- [2] AN061: Optimization of covalent immobilization of Recombinant Prokaryotic Lectins on CIMmic™ platform
- [3] AN060: Preparing small affinity monoliths for antibody purification: Reproducibility of covalent immobilization of recombinant protein A on CIMmic™ CDI-0.1 column
- [4] Immobilization procedures for CDI Monolithic Columns
- [5] Černigoj et al. "Characterization of methacrylate chromatographic monoliths bearing affinity ligands". Journal of chromatography. A, 1464 (2016): 72-78

Ordering information

Catalog No.	Product description
103.8000-2	CIMmic™ CDI-0.1 Disk (Carbonyldiimidazole) (Pores 2 µm) - Pack of 3
103.8001-2	CIMmic™ ALD-0.1 Disk (Aldehyde) (Pores 2 µm) - Pack of 3
103.8002-2	CIMmic™ HDZ-0.1 Disk (Hydrazide) (Pores 2 µm) - Pack of 3
103.8005-2	CIMmic™ Screening Pack - Pack of 3
102.0000	CIMmic™ complete housing
311.1218-2	CIMmultus™ Oligo dT18 - 1 mL (2 µm)
411.1218-2	CIMmultus™ Oligo dT18 - 8 mL (2 µm)
611.1218-2	CIMmultus™ Oligo dT18 - 80 mL (2 µm)
811.1218-2	CIMmultus™ Oligo dT18 - 800 mL (2 µm)

Services

BIA Separations has a commitment to cater for customer's needs in the field of chromatography and CIM monolithic columns. Beside column production, BIA offers immobilization service. Immobilization of antibodies (Abs) is a challenging task. Let us do the hard work for you. For more information please contact our technical support at help@biaseparations.com.

Acknowledgements

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

For any additional information or further enquiries please contact us:

Tel.: +386 59 699 500

help@biaseparations.com
sales@biaseparations.com

www.biaseparations.com