SARTORIUS

Method Guide

Quick Guide for Purification of mRNA With CIM® Octa Oligo dT Columns

Introduction

CIM® Octa columns are miniaturized eight-in-line columns designed for automated and parallel screening of chromatographic conditions on a liquid handling robot with a needle. The columns enable high-throughput process development through fast and efficient evaluation of binding, washing, elution conditions and other chromatographic parameters of large biomolecules such as viruses, nucleic acids, exosomes, bacteriophages, etc. Each miniaturized column is prefilled with a defined amount of monolith that has the same chromatographic properties as our preparative line of chromatographic monolithic columns. CIM Octa columns can be disposable or multi-use and are made from medical grade polypropylene (PP), a material that prevents target molecules from binding to the plastic.

Presented method describes quick and simple purification of mRNA after IVT reaction using CIM® Octa Oligo dT18 0.05 mL Monolithic Column (2 µm channels). Their main advantage is automated high-throughput purification of small mRNA samples.

For more information, contact
help.BIA@sartorius.com

How to Purify mRNA With CIM Octa Oligo dT Column

Buffer preparation (not included with a product):

All work must be carried out in an RNAse-free environment. Handle all reagents, materials and equipment in a manner to prevent contamination with RNases. Buffers and other solutions should be freshly prepared and filtered through a sterile 0.22 µm PES filter. The pH and conductivity of the buffers should be recorded offline.

Always ensure mobile phases are compatible before mixing them or applying consecutively on the column. Examples of in-compatible buffers are magnesium ion-containing buffers and sodium hydroxide (forms precipitate), acetonitrile and sodium hydroxide (forms ammonia and acetate), ethanol and sodium hydroxide (forms ethoxides). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

Table 1: List of buffers needed.

Solution	Label	Composition
Loading Buffer	MPA	50 mM sodium phosphate, 0.5 M NaCl, pH 7.5
Wash Buffer	MPB	50 mM sodium phosphate, pH 7.5
Elution Buffer	MPC	ddH_2O
CIP Buffer	MPD	0.5 NaOH
Storage solution		20 % Ethanol

Note: CIM Octa Oligo dT18 0.05 mL Monolithic Column (2 µm channels) has a column volume (CV) of 0.05 mL.

Removal of storage solution and column conditioning:
Follow guidelines for Octa column operations as in Instructions for use. Gently remove upper and bottom cover seal and place the column in an array plate. Each individual column can be taken from the plastic column holder and placed on the array plate. In total up to 12 eight-in-line CIM Octa columns can be arranged on a 96-array plate. Operating parameters can be found under Technical Data.
Note: To ensure robustly and consistent performance, equilibration should be conducted before each experiment beginning, especially if the plate has been stored, regenerated, or cleaned in place.

Equilibrate the columns:

- Wash the column with 20 CV of MPA. pH and conductivity of passed buffers should match buffer specifications.

Prepare and load sample:

- Prepare sample for application with 10-times dilution with MPA. Load the sample on the column. Collect flow through fraction.

Wash1 with MPA:

- Wash the column with 10 CV of MPA. Collect flow through fraction.

Wash2 with MPB:

- Wash column with 10 CV of MPB to remove non-specifically bound nucleic acids. Collect flow through.

Elution:

- Elute with 3-4 CV. Collect the elution.

Cleaning in Place (CIP) and Storage:

- Replace deep-well collection plate with waste container.
- Perform CIP by washing the column with 10 CV of CIP Buffer (MPD)
- Re-equilibrate the column by washing it with at least 20 CV of Loading Buffer (MPA).
- Wash the column with 10 CV of ddH_2O.
- Wash the column with 10 CV of Storage Solution.
- Seal the column and store it at the temperature as specified in the table Technical Data

Variations, Optimization, and Troubleshooting

The sample precipitated.

Sample in high salt conditions could precipitate, if not processed quickly. Lower salt concentration for that mRNA construct will help to avoid precipitation. Precipitation of mRNA is construct dependent.

I want to transfer my method to preparative CIMmultus® column. What do I need to do?

You can easily transfer the same conditions you obtained on the Octa to CIMmultus format. Same conditions that were used for the process on your Octa format may be used on preparative column format. Some optimization could be required due to different flow characteristics of CIMmultus column.

I want to design an experiment to check multiple parameters. What do I need to do?

You can couple CIM Octa column with MODDE® software. The columns allow for automated processing of up to 96 experiments, leading high consistency and quicker results than manual chromatography methods. With MODDE software you can tackle your toughest challenges in development and manufacturing.

Ordering Information

Cat No.	Product Name
BIA-128.1218-2	CIM® Octa Oligo dT18 0.05 mL Monolithic Column (C6 Linker) (2 µm channels)
BIA-128.1219-2	CIM® Octa Oligo dT18 0.05 mL Monolithic Column (C12 Linker) (2 µm channels)

FAQ

What is the typical binding capacity for mRNA?
Typical binding capacity on the Oligo dT monolith is 2-4 mg per mL of monolith. On a single miniaturized column, you can expect to load mRNA in a capacity of 100-200 µg.

Can I reuse the columns?
Yes. The columns can be cleaned with 0.5 M NaOH. Please refer to the Instructions for use document for cleaning procedure.

Which robotic system does the column work with?
CIM Octa columns were tested on Tecan Fluent and Tecan EVO liquid handlers.

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