SARTORIUS

Method Guide

Purification of mRNA With CIM® Oligo dT Monolithic 96-well Plate

Introduction

CIM® Monolithic Well Plates ensure robust and reliable results for screening multiple chromatographic conditions simultaneously or high-throughput purification of large biomolecules such as viruses, nucleic acids, exosomes, bacteriophages, etc. Each well in our monolithic plates is prefilled with a defined amount of monolith to enable uniform high flow rates across the plate. The monoliths in the wells of the plate have the same chromatographic properties as our preparative line of chromatographic monolithic columns. CIM plates 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. They are manufactured according to ANSI standards and are automation compatible. They can also be operated manually with a vacuum, centrifuge or positive pressure.

Presented method describes quick and simple high-throughput purification of mRNA after IVT reaction using CIM® Oligo dT Monolithic 96-well Plates (2 µm channels). Their main advantage is high-through put purification of mRNA samples where small amount of mRNA is needed.

For more information, contact
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How to Purify mRNA With CIM® Oligo dT 0.05 mL Monolithic 96-well Plate (2 µm channels)

Plate design: Before initiating the design of the 96-well plate experiment, make sure to clearly mark the sample numbers, identify the replicates, and choose the options for the purification gradient.

Table 1: *Example of 96-well plate design for mRNA purification.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1, replicate 1	Sample 2, replicate 1	Sample 3, replicate 1									
B	Sample 1, replicate 2	Sample 2, replicate 2	Sample 3, replicate 2									
C	Sample 1, replicate 3	Sample 2, replicate 3	Sample 3, replicate 3									
F	Sample 1, replicate 1	Sample 2, replicate 1	Sample 3, replicate 1									
E	Sample 1, replicate 2	Sample 2, replicate 2	Sample 3, replicate 2									
F	Sample 1, replicate 3	Sample 2, replicate 3	Sample 3, replicate 3									
G												
H												

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.

Prepare buffers and solutions as described in Table 2. Calculate the required volume of buffers based on the number of experiments and the chosen gradient options.

Table 2: *List of buffers and amount of solutions needed for using all 96 wells.*

Solution	Label	Composition	Volume need for plate (in L)
Loading Buffer	MPA	50 mM sodium phosphate, 0.5 M NaCl, pH 7.5	0.6
Wash Buffer	MPB	50 mM sodium phosphate, pH 7.5	0.2
Elution Buffer	MPC	ddH_2O	0.3
CIP Buffer	MPD	0.5 NaOH	0.1
Storage solution		20 % Ethanol	0.15

Note: volumes calculated for plate with

- Well volume (WV): 0.9 mL
- Monolith volume (MV): 0.05 mL

Removal of storage solution and plate conditioning:
Follow guidelines for plate operations as in Instructions for use. The plate does not require a fully automated robot system and can be operated using a vacuum manifold, positive pressure manifold or a centrifuge. Operating parameters can be found under Technical Data.

It is possible to use limited number of wells in the plate. In this case, remove the top cover lid only for the wells in use to ensure unused wells are still in storage solution. Bottom cover can be removed for the entire plate. Note: To ensure the plate operates robustly and consistently, equilibration should be conducted before each experiment beginning, especially if the plate has been stored, regenerated, or cleaned in place.

1. Before use, remove the top and bottom cover seals and remove storage solution by vacuum or centrifugation. Discard the flow through.
2. Wash out the residual storage solution by adding at least 1 WV of ddH_2O into the selected wells. Discard the flow through.
3. Wash each well with at least 1 WV of Wash buffer (MPB). Discard the flow through.
4. Equilibrate the wells by filling with at least 2-times 1 WV of loading buffer (MPA). Discard the flow-through.

Sample preparation:
- Prepare mRNA samples for loading on the plate with 10-times dilution with MPA.

Sample loading:
- Replace the waste container with a deep-well collection plate
- Load up to 1 WV of sample to pre-conditioned 96-well plate. Repeat loading, if required. Collect flow through fractions.
- Replace the deep-well collection plate with a new one.
- Wash plate with 1 WV of Loading Buffer (MPA). Collect flow through fraction.

Wash plate:
- Replace the deep-well collection plate with a new one
- Wash plate with 1 WV of Wash Buffer (MPB) to remove non-specifically bound nucleic acids. Collect the flow through.

Elution:
- Replace the deep-well collection plate with a new one
- Elute the bound mRNA with up to 1 WV of Elution buffer (MPC). Collect flow through.

Preparing sample for analytics:
- Transfer 100 µL of the elution fractions from the deep-well collection plate to transparent 96-well microtiter plate for UV260 absorbance measurements. Dilute the eluate with 100 µL of ddH_2O. Prepare a calibration curve for quantification.

Cleaning in Place (CIP) and Storage:
- Replace the deep-well collection plate with waste container.
- Perform CIP by washing the wells with 1 WV of CIP Buffer (MPD).
- Re-equilibrate the wells by washing them with at least 2 WV of Loading Buffer (MPA).
- Wash the wells with 1 WV of ddH_2O.
- the wells with 1 WV of Storage Solution.
- Add storage solution to each well.
- Seal the plate and store it at the temperature as specified in the table Technical Data.

Variations, Optimization, and Troubleshooting

I need larger amount of mRNA purified in high-throughput manner.
Oligo dT chemistry is available also in 96-well monolithic plates with 0.2 mL monoliths, and 24-well plates with 1.0 mL monoliths inside each well. Same protocol can be used with both plate sizes.

The sample precipitated in the well.
Sample in high salt conditions could precipitate, if not processed quickly. Due to mode of operation the wells once clogged are more difficult to clean and restored to full performance. 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 plate to CIMmultus® format. Same conditions that were used for the process on your plate may be used on preparative column format. Some optimization could be required due to different flow characteristics of CIMmultus® column.

Ordering Information

Cat No.	Product Name
BIA-122.1218-2	CIM® Oligo dT18 0.05 mL Monolithic 96-well Plate (C6 Linker) (2 µm channels)
BIA-122.1219-2	CIM® Oligo dT18 0.05 mL Monolithic 96-well Plate (C12 Linker) (2 µm channels)
BIA-120.1218-2	CIM® Oligo dT18 0.2 mL Monolithic 96-well Plate (C6 Linker) (2 µm channels)
120.1219-2	CIM® Oligo dT18 0.2 mL Monolithic 96-well Plate (C12 Linker) (2 µm channels)
BIA-124.1218-2	CIM® Oligo dT18 1 mL Monolithic 24-well Plate (C6 Linker) (2 µm channels)
BIA-124.1219-2	CIM® Oligo dT18 1 mL Monolithic 24-well Plate (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 the plates you can expect to load mRNA in a capacity of:

- 100-200 µg per well (BIA-122.1218-2; BIA-122.1219-2)
- 400-800 µg per well (120.1219-2; BIA-120.1218-2)
- 2-4 mg per well (BIA-124.1218-2; BIA-124.1219-2).

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

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