Instructions for Use

CIM® Oligo dT18 1 mL Monolithic 24-well Plate (C6 Linker) (2 µm channels)

CIM Convective Interaction Media®
BIA-124.1218-2

SARTORIUS

Contents

1. About These Instructions for Use

These instructions are part of the device. They apply to the device product number indicated on the cover page.

2. Safety

⚠WARNING
Denotes a hazard that may result in death or severe injury if it is not avoided.

⚠CAUTION
Denotes a hazard that may result in moderate or minor injury if it is not avoided.

NOTICE
Denotes a hazard that may result in property damage if it is not avoided.

2.1. Intended Use

CIM® monolithic plates are standard format plates prefilled with unique monolithic chromatographic media intended for diagnostic or screening purposes. They enable high-throughput process development through fast and efficient evaluation of binding, washing, elution conditions and other chromatographic parameters. The properties of the medium are directly comparable to CIM® chromatographic columns, making monolithic plates a robust tool in early process development stages.

CIM® Oligo dT monolithic plates are used for fast, highly efficient screening of samples or purification parameters for RNA with a poly-adenylated tail. The following information is being provided to ensure proper product care and optimal product performance.

2.2. Safety Note

Follow the guidelines in this Instructions for Use. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow safety instructions, wear gloves, safety glasses, and a lab coat during operation.

3. Technical Data

Column chemistry	Oligo dT18 coupled to CDI-activated matrix, C6 Linker
Channel radius	1050 nm (950 nm – 1150 nm)
Support matrix	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)

Monolith dimensions	Diameter: 14.8 mm; length: 5.8 mm; monolith volume: 1 mL
Plate format	127 x 85.5 x 44 mm, plate material: polypropylene (PP) and polyethylene (HDPE)
Well load volume (WV)	Up to 6000 µL, 24 wells
Operating parameters	Operating vacuum between -0.015 and -0.03 MPa | -0.15 and -0.3 bar | -2.18 and -4.35 psi; Maximum vacuum -0.06 MPa | -0.6 bar | -8.7 psi; Operating centrifugation force 500 g; Maximum centrifugation force 1000g; Operating positive pressure between 9 and 11 psi; Maximum positive pressure 15 psi.
Operating temperature	4 °C (39 °F) to 30 °C (86 °F)
Chemical stability	All commonly used aqueous buffers, sodium hydroxide (short term up to 0.5 M, see cleaning guidelines), 6 M guanidine hydrochloride, 12 M guanidine thiocyanate, 10 M urea, 20 % ethanol.
Recommended pH	Working range 2-10, Cleaning in place 2–13
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); 20 % ethanol
Shelf life	3 years

4. Installation

Remove the product from its shipping box or crate and place on a flat surface. Carefully inspect the product for any damage that may have occurred during shipping. Immediately report any such damage to your vendor and the courier. The product is shipped in the designated storage solution at ambient temperature and should be stored upon receiving as stated under Technical Data.

NOTICE
Do not store the product below 0 °C (32 °F).

5. Getting Started

The plate does not require a fully automated robot system and can be operated using a vacuum manifold or centrifuge. Operating parameters can be found under Technical Data. Before use, remove the top and bottom cover seals and remove storage solution by vacuum or centrifugation.

Note: When using a vacuum manifold with a vacuum pump, the maximum separation between the bottom of the plate and the top of the collection plate should not exceed 5 mm to prevent cross contamination. Turn off the vacuum as soon as the sample/solution is removed.
Note: With a centrifuge, ensure the collection plate well volume is suitable for the intended loading volume. Centrifuge the plate until the sample/solution is removed. Adjust centrifugation time accordingly.

5.1. General Recommendations

The following are general guidelines to consider when working with chromatography. The guidelines may not apply to

specific plate chemistry or sample properties.

- Treat loading material appropriately (e.g. pre-treat, filter, concentrate / dilute, etc.). For more details, please refer to the Guideline 'Pre-treatment of complex biological samples before column purification and regeneration procedures for columns with increased back pressure' (biaseparations.com/en/library/guidelines).
- Always use freshly prepared mobile phases, filtered through 0.2 µm filter, compatible with mobile phases.
- Air bubbles will not disturb the stationary phase and can be washed out of the plate. However, drying the monolith risks damaging the stationary phase.
- Surfactants can improve recoveries in virus purification. Non-ionic surfactants will not interact with ion exchange chromatography media. Non-UV-absorbing (at working wavelengths) surfactants will improve the baseline signal.
- Ensure all components of the system used are compatible with the working solutions (e.g. sodium hydroxide, organic solvents, high salt concentrations, etc).

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

5.2. Buffer Selection

Oligo dT binds poly-adenylated RNA by hydrogen bonding interaction between the nucleotide residues in the presence of salt. Salts suppress negative charge repulsion between RNA and the ligand and allow formation of a stable hybrid. Detailed process optimisation parameters can be found in the Accompanying Documents, simple guidelines are outlined here.

The product is compatible with commonly used biological buffers (Tris, BTP, HEPES, etc). Addition of EDTA (up to 20 mM) in all buffers will minimise non-specific effects of multivalent metal cations and is recommended.

Parameters which change the protonation of biomolecules will affect the retention of the sample. A neutral pH is a suitable starting point. Lower pH increases the protonation of nucleotide residues, which results in stronger binding. pH is temperature dependent, and changes in binding or elution performance can be expected without adequate temperature control.

Any salt that precipitates RNA will promote binding. Neutral salts (e.g. sodium, potassium chloride or acetate) are preferred over kosmotropic salts (phosphates, sulfates), which may induce non-specific associations and affect the contaminant level in the product. Excess binding salt may increase binding capacity but it risks non-specific binding and precipitation of RNA. The salt type and concentration can be evaluated experimentally. As a starting point, a range of 250 mM - 1.5 M NaCl can be tested.

6. Operating the Plate

6.1. Equilibration

For robust and consistent operation of the plate, equilibration should be performed before starting with sample analysis when the plate was stored, regenerated or cleaned in place. Flush any storage or cleaning solution out of

each well by washing with 2 WV of deionised water. Equilibration buffer should have the same or similar composition to the loaded sample.

1. If needed wash each well with 2 WV of water to prevent mixing of incompatible buffers.
2. Wash each well with at least 3 WV of elution mobile phase.
3. Wash each well with at least 2 WV of binding mobile phase. The composition of this mobile phase should be similar to the sample composition.

7. Cleaning | Maintenance

Cleaning and maintenance of the device may improve its lifetime and increase reproducibility. Sample properties should be taken into account for plate cleaning.

7.1. Cleaning in Place (CIP)

Cleaning of plate wells is recommended between consecutive sample applications. A reduced flow rate is suggested for plate cleaning to extend contact time with the cleaning and neutralisation-equilibration solutions. Lower pressure or lower centrifugal force can be used to extend the cleaning time (-0.2 Bar or 500 g).

⚠**CAUTION**
Remain inside operating parameters specified in Technical Data.

⚠**CAUTION**
Ensure compatibility between the current plate solution and cleaning solutions (see examples in General Recommendations).

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1. If needed wash each well with 2 WV of water to prevent mixing of incompatible buffers.
2. Wash each well with at least 2 WV of cleaning solution with 0.5 M NaOH. Contact time of up to 30 min is recommended.
3. Wash each well with 2 WV of water.
4. Wash each well with at least 2 WV of a neutralisation-equilibration solution. A buffer (e.g. Tris pH 7) with high salt concentration is recommended (e.g. binding mobile phase). A solution of 1 M ammonium acetate may be used. **Note:** Collect ammonium acetate solution in a separate waste container to avoid mixing with NaOH.

To improve cleaning, increase the WV in step 2 or implement cleaning steps specific to the contaminants present in the sample.

8. Storage

Wash the plate with at least 2 WV per well of deionized water and proceed with at least 2 WV per well of storage solution. Add storage solution to each well. Seal the plate and store at the temperature specified in the table Technical Data. If there is a possibility of biological contamination from the sample it is recommended to store the plate between 2 °C (36 °F) and 8 °C (46 °F).

Note: Clean and equilibrate the plate before long-term storage.
Note: NaOH-ethanol mixtures at any concentration form ethoxide anions that are highly destructive to biomolecules. Caution is recommended. Neutralise the plate environment before introducing ethanol.

9. Troubleshooting

Problems arising during the analysis are usually related to the device, sample, mobile phase, or the instrumentation. It is advisable to use an elimination approach to exclude possible causes. Please refer to our troubleshooting guide (biaseparations.com/en/library/guidelines).

10. Decommissioning | Transportation

If there is reason to return the product, complete a Return Form (biaseparations.com/en/terms-conditions) and contact help.bia@sartorius.com.

Contaminated samples used during the process that could cause biological or chemical hazards are potentially hazardous substances. If the product has come into contact with hazardous substances, steps must be taken to ensure proper decontamination and declaration.

Procedure
Decontaminate the product. The operator of the product is responsible for adhering to local government regulations on the proper decontamination and declaration for transport and disposal.

11. Ordering Information

Transferring the workflow to a different scale or format (analytical, screening) is simple with CIM®. Contact your local support to find the appropriate products.

Purification Scale Products

Catalog number	Product name
311.1218-2	CIMmultus® Oligo dT18 1 mL Monolithic Column (C6 Linker) (2 µm channels)
414.1218-2	CIMmultus® Oligo dT18 4 mL Monolithic Column (C6 Linker) (2 µm channels)
411.1218-2	CIMmultus® Oligo dT18 8 mL Monolithic Column (C6 Linker) (2 µm channels)
614.1218-2	CIMmultus® Oligo dT18 40 mL Monolithic Column (C6 Linker) (2 µm channels)
611.1218-2	CIMmultus® Oligo dT18 80 mL Monolithic Column (C6 Linker) (2 µm channels)
814.1218-2	CIMmultus® Oligo dT18 400 mL Monolithic Column (C6 Linker) (2 µm channels)
811.1218-2	CIMmultus® Oligo dT18 800 mL Monolithic Column (C6 Linker) (2 µm channels)
1014.1218-2	CIMmultus® Oligo dT18 4000 mL Monolithic Column (C6 Linker) (2 µm channels)
1011.1218-2	CIMmultus® Oligo dT18 8000 mL Monolithic Column (C6 Linker) (2 µm channels)

Sartorius BIA Separations d.o.o.
Mirce 21
SI-5270 Ajdovščina
Phone +386 59 699 500
www.biaseparations.com

The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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