

Instructions for Use

# CIMmultus<sup>®</sup> Oligo dT18 8000 mL Monolithic Column (C6 Linker) (2 $\mu$ m channels)

CIM Convective Interaction Media<sup>®</sup>  
1011.1218-2



**SARTORIUS**

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# 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.

## 1.1. Accompanying Documents

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Column integrity test



Starting conditions for purification of mRNA with  
Oligo dT18



## 2. Safety

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### **⚠ WARNING**

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

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### **⚠ CAUTION**

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

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### **NOTICE**

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

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## 2.1. Intended Use

CIMmultus<sup>®</sup> Monoliths are reusable chromatography devices for scalable high-resolution purification of complex biological samples. Inside the custom designed housing is a single-piece stationary phase with homogeneous channel size and surface chemistry. Without the need for column packing, CIMmultus<sup>®</sup> Monoliths are ready for use out of the box.

Oligo dT columns are primarily intended for fast and efficient capture and purification of messenger RNA with a poly-

adenylated tail, from various sources. The following information is 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

<b>Column chemistry</b>	Oligo dT18 coupled to CDI-activated matrix, C6 Linker
<b>Channel radius</b>	1050 nm (950 nm - 1150 nm)
<b>Support matrix</b>	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
<b>Monolith dimensions</b>	Outer diameter: 300 mm; inner diameter: 255 mm; length: 415 mm; bed volume (CV): 8000 mL
<b>Connector</b>	TC 1 in. (25 mm), 8 mm ID bore
<b>Ligand density</b>	> 0.5 mg Oligo dT/mL wet support
<b>Operating flow rates</b>	Up to 1 CV/min   8000 mL/min   135 cm/h. Do not go below 0.1 CV/min
<b>Maximum pressure</b>	0.7 MPa, 7 bar, 100 psi
<b>Operating temperature</b>	4 °C (39 °F) to 30 °C (86 °F)
<b>Chemical stability</b>	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.
<b>Recommended pH</b>	Working range 2-10, Cleaning in place 2-13
<b>Storage conditions</b>	2 °C (36 °F) to 25 °C (77 °F); 20 % ethanol
<b>Shelf life</b>	3 years

The linear flow rate can be calculated with the following equation and supporting data, which is available in the Technical Data.

$$\text{Average linear velocity, } u_{av} = \frac{F}{\pi \times L} \frac{\ln\left(\frac{D_o}{D_i}\right)}{(D_o - D_i)}$$

F is the flow rate in mL/min, Do and Di are the outer and inner diameter of the column and L is the column length.

## 4. Device Overview | Description

The housing of this CIMmultus® column is made of epoxy thermoset material. Its surface is coated pinhole-free with

biocompatible (USP Class VI) Parylene C.

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**NOTICE**

Do not expose the column housing to pure acetone.

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## 5. 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.

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**NOTICE**

Larger columns are shipped in a wooden crate, and a suitable stand is provided in the packaging. The columns have either a stand (400 | 800 mL columns) or wheels (4 | 8 | 40 L columns). Place them in an upright position on a flat surface. The 40 L column should be lifted from its crate by attaching straps to the lifting eye bolts on the housing.

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**NOTICE**

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

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## 6. Getting Started

Set the pressure relief valve to the maximum pressure allowed on the CIM column as indicated in Technical Data. Before using the column, an integrity test must be performed. Guideline 'Column integrity test' ([biaseparations.com/en/library/guidelines](https://biaseparations.com/en/library/guidelines)) should be followed. It is advised to repeat this procedure regularly or when deviations in performance are observed.

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**NOTICE**

The column should be equilibrated to working temperature for optimal results. Allow at least 12 h for the column to reach working temperature.

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### 6.1. General Recommendations

The following are general guidelines to consider when working with chromatography. The guidelines may not apply to specific column 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](https://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 column. 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).

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## NOTICE

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), ammonium acetate and sodium hydroxide (potential formation of explosive atmosphere), ethanol and sodium hydroxide (forms ethoxides). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

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## 6.2. 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.

## 7. Operating the Column

### 7.1. Connecting the Column

Position the column with the inlet at the bottom and outlet at the top by placing the 400 | 800 mL column on its stand. The 4000 | 8000 mL columns should stand upright on its wheels. Connect the column to the system with flow turned off in the following order:

1. Carefully remove the blind fitting on the inlet side and connect the inlet tubing.
2. Carefully remove the blind fitting on the outlet side and connect the outlet tubing.

Disconnect by reversing the steps above.

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#### NOTICE

Do not open both inlet and outlet simultaneously to avoid leaking of mobile phase. Changing the order of the above procedure might cause leakage of the mobile phase from the column and affect its performance!

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#### NOTICE

Reversing the flow direction will damage the column. Make sure the column is connected according to the flow direction indicated by the arrow. The 40 L housing has an integrated non-return valve at the column outlet to prevent reversing the flow direction. Do not remove or disassemble the valve. **Note:** Software specific settings which regulate the flow direction should be checked. Ensure the correct flow mode is selected so that flow can go only in the direction indicated on the monolith.

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#### NOTICE

Spikes in pressure generated during sudden pump fluctuations (e.g. immediate application of maximum flow rate or sudden pump stop at high operating pressure) can generate a backpressure shock, which can damage the monolith.

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## 7.2. Equilibration

The column should be equilibrated before use. Equilibration mobile phase should be of same or similar composition to the sample.

1. If needed, wash the column with 10 CV of water to prevent mixing of incompatible buffers.
2. Wash the column with at least 10 CV of elution mobile phase.
3. Wash the column with at least 10 CV of binding mobile phase.

Use system detectors as indication of successful equilibration. Conductivity and pH at the outlet should match buffer specifications.

## 8. Cleaning | Maintenance

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

### 8.1. Cleaning in Place (CIP)

Column cleaning is recommended between purification runs or cycles. A reduced flow rate is suggested for column cleaning to extend contact time with the cleaning and neutralisation-equilibration solutions (between 0.1 and 0.5 CV/min).

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#### CAUTION

Remain below the maximum pressure specified in Technical Data.

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**⚠ CAUTION**

Ensure compatibility between the current column solution and cleaning solutions (see examples in General Recommendations).

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1. If needed wash the column with 10 CV of water to prevent mixing of incompatible buffers.
2. Wash the column with at least 10 CV of 0.5 M NaOH. A contact time of up to 30 min is recommended.
3. Wash the column with 10 CV of water.
4. Wash the column with at least 10 CV 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, extend the contact time with cleaning solution or implement cleaning steps specific to the contaminants present in the sample.

## 8.2. Sanitisation

Use the cleaning in place procedure as starting point for column sanitisation.

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**⚠ CAUTION**

Ensure compatibility between the current column solution and cleaning solutions. Add a 10 CV water wash to prevent mixing if needed. See General Recommendations for frequent incompatible combinations.

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## 9. Storage

Clean and equilibrate the column before storage. The column can be stored in working buffers overnight.

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**NOTICE**

NaOH-ethanol mixtures at any concentration form ethoxide anions that are highly destructive to biomolecules, and ligands on chromatography media. Neutralise the column environment before introducing ethanol.

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1. Wash the column with 10 CV deionised water.
2. Wash the column with 10 CV of storage solution. **Note:** Reduce the flow rate when using viscous solvents (such as ethanol) to avoid a pressure increase.
3. Seal the column with blind fittings and store at the temperature specified in Technical Data. If there is a possibility of biological contamination from the sample it is recommended to store the column between 2 °C (36 °F) and 8 °C (46 °F).

## 10. Troubleshooting

Problems arising during the analysis are usually related to the column, 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](https://biaseparations.com/en/library/guidelines)).

## 11. Decommissioning | Transportation

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

## 12. 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 cGMP Compliant

Catalog number	Product name
BIA-904.1218-2	CIMmultus® Oligo dT18 4 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
901.1218-2	CIMmultus® Oligo dT18 8 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
914.1218-2	CIMmultus® Oligo dT18 40 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
911.1218-2	CIMmultus® Oligo dT18 80 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
924.1218-2	CIMmultus® Oligo dT18 400 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
921.1218-2	CIMmultus® Oligo dT18 800 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
934.1218-2	CIMmultus® Oligo dT18 4000 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)
931.1218-2	CIMmultus® Oligo dT18 8000 mL cGMP Compliant Monolithic Column (C6 Linker) (2 µm channels)

**Purification Scale Products non-cGMP Compliant**

<b>Catalog number</b>	<b>Product name</b>
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)



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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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