

## Instructions for Use

# CIM<sup>®</sup> r-Protein G LLD 0.05 mL Monolithic 96-well Plate (recombinant Protein G, low ligand density) (2 $\mu$ m channels)

CIM Convective Interaction Media<sup>®</sup>  
BIA-122.1012-2



# 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.

## 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

CIM<sup>®</sup> 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<sup>®</sup> chromatographic columns, making monolithic plates a robust tool in early process development stages.

CIM<sup>®</sup> r-Protein G monolithic 96-well plates are used for fast, highly efficient screening of immunoglobulin G samples purified from cell culture supernatant or human plasma. The antibody binds to the immobilised protein through the Fc portion, leaving the antigen binding site free. The range of polyclonal IgGs that bind strongly to protein G is wider than on protein A and adsorption takes place over a wider pH range. 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>	r-Protein G (affinity; recombinant protein G produced from E. Coli coupled to CDI-preactivated support – low ligand density)
<b>Channel radius</b>	1050 nm (950 nm – 1150 nm)
<b>Support matrix</b>	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
<b>Monolith dimensions</b>	Diameter: 5 mm; length: 2.5 mm; monolith volume: 0.05 mL
<b>Plate format</b>	127 x 85.5 x 31 mm, plate material: polypropylene (PP) and polyethylene (HDPE)
<b>Well load volume (WV)</b>	Up to 900 µL, 96 wells
<b>Operating parameters</b>	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.
<b>Dynamic binding capacity</b>	≥ 10 mg hlgG/mL wet support , Human IgG: octagam® 5% (Octapharma®)
<b>Operating temperature</b>	4 °C (39 °F) to 40 °C (104 °F)
<b>Chemical stability</b>	All commonly used aqueous buffers, 8 M urea, 6 M guanidine hydrochloride and 20 % ethanol solution.
<b>Recommended pH</b>	Working range 2–11, cleaning in place 1–13
<b>Storage conditions</b>	2 °C (36 °F) to 8 °C (46 °F); 20 % EtOH in 20 mM Tris pH 7.4
<b>Shelf life</b>	2 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](http://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).

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### 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.

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## 5.2. Buffer Selection

The most commonly used bind/wash buffers are sodium phosphate buffer and TRIS buffer (25 to 100 mM; 50 mM is recommended as starting concentration). Other buffers with pH between 6 and 9 can be used (pH 7.4 is recommended as a starting point). Device is compatible with neutral salts (e.g. sodium chloride or potassium chloride). Salt concentrations of 0.1 to 0.2 M can improve the peak shape and/or solute recovery.

Elution should be performed under low pH conditions using a suitable buffer. The most commonly used buffers are citric acid, glycine and acetic acid. The table below summarizes these buffers, their concentration and pH range.

Table 2: Elution buffers and their pH range.

Buffer	Concentration	pH range
Glycine	0.1 M	2.0-3.0
Citric acid	0.1 M	2.5-3.0
Acetic acid	5-20%	2.5-3.0

## 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. Flush any storage or cleaning solution out of the plate by washing each well with 1 WV of deionised water. The plate should be equilibrated as follows:

1. Wash each well with at least 1 WV of the binding mobile phase at the operating flow rate.
2. Wash each well with at least 1 WV of the eluting mobile phase.
3. Finally, wash each well with at least 1 WV of the binding mobile phase again.
4. The plate is now ready for use.

### 6.2. Strip | Regeneration

Regeneration of the plate: removal of ionically bound compounds from the monolith, followed by re-equilibration:

1. Flush each well with at least 2 WV of 0.1 M buffer containing 1.0 M NaCl, pH 7–8 at the operating flow rate.
2. Flush each well with at least 2 WV of concentrated elution buffer (e.g. 0.1 M glycine, pH 2.0).
3. Re-equilibrate each well with at least 2 WV of binding mobile phase.

Regeneration of the plate: removal of hydrophobically bound compounds from the monolith, followed by re-equilibration:

1. Flush each well with at least 2 WV of 0.1 M buffer, pH 7–8 at the operating flow rate.
2. Flush each well with at least 2 WV solution of ethanol and buffer (20 mM TRIS, pH 7.4) in the ratio 1:4 (v/v).
3. Re-equilibrate each well with at least 2 WV of binding mobile phase.

To maintain the optimal efficiency of the plate, it is advisable to perform the regeneration after each sequence of sample analyses.

## 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)

In some cases, a simple regeneration of the plate is not sufficient. Sample molecules may not completely elute from the plate or may even precipitate on the plate. This build-up of contaminants on the monolithic plate may cause loss of resolution and binding capacity or a complete blockage of the plate. A specific CIP procedure should be designed for the type of contaminants present in the sample. In most cases the following procedure can be used:

Removal of precipitated proteins

1. Wash each well with 1 WV of 0.1 M NaOH.
2. Wash each well with 1 WV of distilled water.

3. Wash each well with 1 WV of a concentrated buffer (e. g. 0.1–0.5 M buffer) in order to restore the appropriate pH.
4. Re-equilibrate each well with 1 WV of the working mobile phase (buffer).

Removal of strongly bound hydrophobic proteins or lipids

1. Wash each well with 1 WV of distilled water.
2. Wash each well with 1 WV of 30 % 2-propanol.
3. Wash each well with 1 WV of distilled water.
4. Re-equilibrate each well with 1 WV of the working mobile phase (buffer).

## 8. Storage

Wash the plate with at least 1 WV per well of deionized water and proceed with at least 1 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](https://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](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.

# 11. Ordering Information

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



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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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