Data Sheet

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# Agilent Bio-Monolith Protein A, rProtein A, and Protein G Columns

## Characteristics and shipping conditions

The information in this data sheet is provided to ensure proper product care and maximum product lifetime.

| | |
|---|---|
| Catalog Number | Agilent Bio-Monolith Protein A (p/n 5069-3639)<br>Agilent Bio-Monolith rProtein A (p/n 5190-6903)<br>Agilent Bio-Monolith Protein G (p/n 5190-6900) |
| Immobilized Ligand<br>Agilent Bio-Monolith Protein A<br>Agilent Bio-Monolith rProtein A<br>Agilent Bio-Monolith Protein G | <br>Immunoaffinity Protein A from *Staphylococcus aureusis*<br>Immunoaffinity r-Protein A from *Escherichia coli*<br>Immunoaffinity r-Protein G from *Escherichia coli* |
| Support Matrix | Poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith |
| Column Dimensions | Diameter 5.2 mm, length 4.95 mm, bed volume (CV) 0.10 mL |
| Dynamic Binding Capacity<br>Agilent Bio-Monolith Protein A<br>Agilent Bio-Monolith rProtein A<br>Agilent Bio-Monolith Protein G | <br>>8 mg hIgG/mL wet support<br>>5 mg hIgG/mL wet support<br>>9 mg hIgG/mL wet support<br>(Conditions: hIgG 0.5 mg/mL, PBS buffer, pH 7.4, flow rate 1 mL/min) |
| Maximum Loading Capacity | 0.4 to 0.5 mg |
| Working Flow Rates | Recommended: 0.2 to 2 mL/min (1 to 10 cm/min, 2 to 20 CV/min)<br>Maximum: 3 mL/min (15 cm/min, 30 CV/min) |
| Maximum Allowed Pressure Over the Column | 150 bar (15 MPa, 2,100 psi)<br>**WARNING:** Do not exceed the maximum allowed pressure, as this might seriously damage your column. |
| Temperature Stability | Working 4 to 40 °C Storage 4 to 8 °C<br>**WARNING:** Avoid prolonged use at elevated temperatures. |
| Recommended pH* | Working range: 2 to 11<br>Cleaning in place: 2 to 13 |
| Shipping Solvent | 20 mM Tris, pH 7.4 and 20 % ethanol |

* At high pH, ligands can slowly degrade, therefore, only use pH higher than 11 for regeneration, cleaning-in-place, and sanitization procedures.

Agilent Bio-Monolith Protein A, rProtein A, and Protein G columns are analytical affinity columns designed for fast separation, isolation, and quantitation of antibodies in fermentation (cell culture supernatants and lysates). The columns are compatible with HPLC and preparative LC systems. The monolithic structure enables high reproduciblity and long lifetimes for separation and quantitation of immunoglobulin G (IgG) at high speeds.

## Preparing the column before first use

### Connecting the column

The flow direction is indicated in Figure 1. The flat end is the inlet, and the pointed end is the outlet.

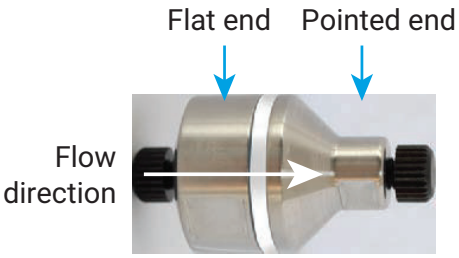


**Figure 1.** Flow direction through the flat end of the column.

1. Remove both end caps.
2. Connect the flat end* to the nut of the capillary, which is already connected to the HPLC system.
3. Tighten the fitting as instructed by the manufacturer.
4. Flow liquid into the column.
5. Connect outlet (pointed end) to the detector.

* Long to extra long fittings and ferrules (p/n 5065-9967) should be used.

### Column equilibration

1. The columns should be washed with at least 2 mL (20 CV) of a 0.1 M buffer such as sodium phosphate buffer or Tris buffer containing 1 M NaCl, pH 7.0 to 8.0 at a flow rate of 0.5 to 1.0 mL/min.
2. Wash the columns with at least 2 mL (20 CV) of eluting buffer (see eluting buffers below).
3. Equilibrate the column with 30 CV of the binding buffer (see binding and washing buffers below) at a flow rate of 0.5 to 1.0 mL/min.
4. The column is ready for injection.

### Binding and washing buffers

Bio-Monolith Protein A, rProtein A, and Protein G columns are compatible with many buffers for binding and washing. The most common ones are:

1. Sodium phosphate buffer and Tris buffer, 25 to 100 mM. We recommend using 50 mM as the starting concentration.
2. Binding and washing buffers with pH 6 to 9. We recommend starting with pH 7.4. This pH seems to be the optimal for the columns to absorb/bind most antibodies.
3. Room temperature or 24 to 25 °C is recommended for optimal performance and extended column lifetime. Higher temperature can be also used, but might reduce column lifetime.
4. The columns are compatible with neutral salts such as sodium chloride (NaCl) or potassium chloride (KCl). Salt concentrations of 0.1 to 0.2 M can improve the peak shape and recovery in some cases for some samples.

**Note:** Filtration of buffers through a 0.22 or 0.45-µm membrane is recommended to reduce buffer impurities that build up on the frits inside the column. This will prevent column blockage.

**Note:** Binding/washing buffers should be freshly made.

### Eluting buffers

Bio-Monolith Protein A, rProtein A, and Protein G are compatible with many low pH buffers. The buffers commonly used are citric, glycine, HCl, and acetate acid. Table 1 provides details for these commonly used buffers.

**Table 1.** Some common eluting buffers for use with Agilent Bio-Monolith Protein A, rProtein A, and Protein G columns.

| Column | Buffer | Conc. | pH |
|---|---|---|---|
| Agilent Bio-Monolith Protein A and rProtein A | Citric acid | 0.1 M | 2.5 to 3.0 |
| | Glycine | 0.1 M | 2.5 to 3.0 |
| | Acetic acid | 5 to 20 % | |
| Agilent Bio-Monolith Protein G | Citric acid | 0.1 M | 2.0 to 2.5 |
| | Glycine | 0.1 M | 2.0 to 2.5 |
| | Acetic acid | 5 to 20 % | |

**Note:** Commonly, elution buffers for affinity columns have a refractive index (RI) very different from binding/washing buffers; therefore, baseline noise and an artifact peak could appear when the eluents start flowing. This peak could interfere with the quantitation of low concentration samples. To minimize this effect, high-quality chemicals are recommended to be used and blank runs should be included to establish the artifact peak. Blank runs can be used for baseline subtraction if desired.

Note: HCl has a lower RI compared to other eluents. If a low concentration sample is used and baseline noise and artifact peaks are of concern, HCl can be used as an eluent.

## Sample preparation

Bio-Monolith Protein A, rProtein A, and Protein G columns are compatible with many buffers. However, some minor preparation is required before injection to optimize column performance and extend column lifetimes.

Centrifuge or filter samples to remove host cell debris and particulates from the supernatant or lysate, to prevent blockage of the columns.

## Optimization/ re-optimization

Please keep in mind that we provide some general guidelines on how to use the columns. Optimization of methods might be needed for some samples. Methods from existing protocols with other affinity columns might not work with Agilent columns due to differences in chemistry between columns and differences between the antibody sources. Therefore, method optimization might be required.

Optimization methods include sample preparation, buffer compositions, buffer pH, binding time, washing time, and eluting time.

## Taking care of the column

### Cleaning/washing the columns between injections

It is a good practice to monitor column performance and include regular column cleaning cycles.

### Cleaning-in-Place (CIP) protocol

1. Wash the column with 1 to 2 mL (10 to 20 CV) 0.1 M NaOH.

   **Note:** Reverse the flow direction and use flow rates between 0.2 to 0.5 mL/min
2. Wash the column with 1 to 2 mL (10 to 20 CV) deionized (DI) water at the working flow rate.
3. Wash the column with 1 to 2 mL (10 to 20 CV) concentrated buffer (for example, 0.1 to 0.5 M phosphate buffer, pH 7.4) to quickly restore the appropriate pH.
4. Equilibrate with at least 5 mL (50 CV) of binding phase buffer at the working flow rate.

If the impurities are highly hydrophobic or lipidic, and are not easily removed from the column, 2-propanol (up to 30%) or guanidine hydrochloride (up to 3 M) can be used to remove these impurities. After using these alternative cleaning solutions, follow steps 1 through 4.

**WARNING:** When you wash the column with these cleaning solutions, always decrease the flow rate on the column to avoid generation of high pressures that might exceed the maximum allowed pressure over the column.

### Short-term storage

For storage overnight or for a few days, the columns can be flushed with binding buffer, disconnected from the instrument, capped, and stored at 4 to 8 °C. Columns should be equilibrated before the first injection after short-term storage.

### Long-term storage

If the column will not be in use for more than 2 days, it should be washed with at least 1 mL (10 CV) of DI water and afterwards flushed with at least 2 mL (20 CV) of 20 % ethanol with 20 mM Tris buffer, pH 7.4 at a flow rate of 0.2 to 0.5 mL/min. It should then be sealed with column end stops and stored at 4 to 8 °C (39 to 46 °F).

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