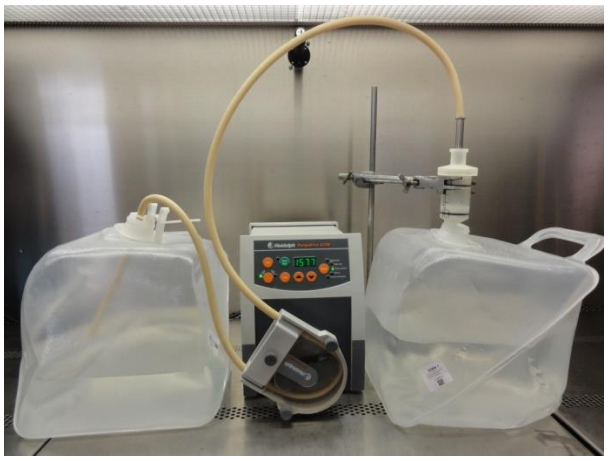
TECHNISCHE UNIVERSITÄT MÜNCHEN

# Monolithic Adsorption Filtration

# User Manual



Version 5

| Date | Name | Commentary |
|---|---|---|
| 23.06.2014 | Dennis Elsaesser | V.1 |
| 08.04.2015 | Dennis Elsaesser | V.2 |
| 15.10.2015 | Dennis Elsaesser | V.3 |
| 09.11.2015 | Dennis Elsaesser | V.4 |
| 11.12.2015 | Dennis Elsaesser | V.5 |

Technische Universität München
Institute of Hydrochemistry & Chair for Analytical Chemistry
Marchioninistr. 17
81377 München
http://www.ws.chemie.tu-muenchen.de/

**Contact:**
Dr. Michael Seidel
Head of Group Bioseparation & Microarrays
Institute of Hydrochemistry & Chair for Analytical Chemistry
Technische Universität München
Marchioninistraße 17
81377 München
Phone: 089/2180-78238
Fax: 089/2180-78255
email: Michael.Seidel@ch.tum.de
http://www.ws.chemie.tu-muenchen.de/groups/seidel

# Table of Contents

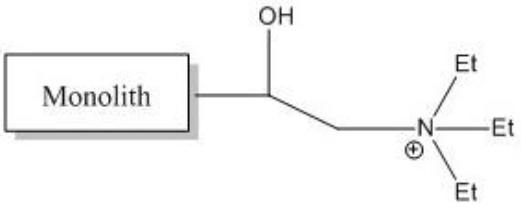
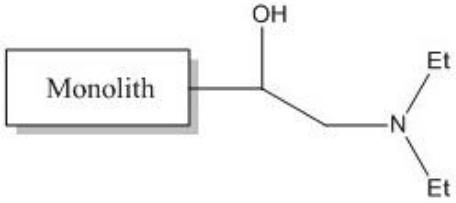
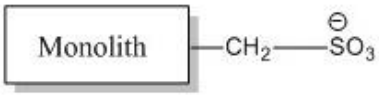
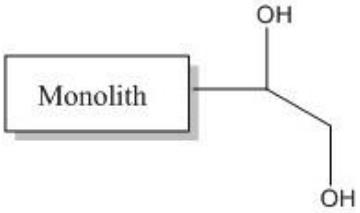
## About MAF Columns

The preparation protocol for Monolithic Adsorption Filtration (MAF) columns is based on the work of Peskoller et al.[1] To synthesize the MAF column the monomer polyglycerol-3-glycidyl (Polypox R9) is polymerized. The polymerization reaction is catalyzed by boron trifluorid diethyl etherate ($BF_3$ $Et_2O$). To form pores in the monomer, a mixture of toluene and tert-butyl methyl ether is added.

Adsorption of microorganisms and viruses to the MAF column is based on electrostatic interaction. Both microorganisms and viruses have a characteristic surface charge, which depends on the pH value of the surrounding matrix. The principle of the MAF process is to bind charged microorganisms and viruses to an oppositely charged MAF-Disk and afterwards elute them by change of the pH value with an elution buffer. For example, bacteriophage MS2 has an isoelectric point (PI) of 3.9. In a matrix with a pH value above 3.9 it is charged negatively and will adsorb to a positively charged surface. To elute it, an elution buffer with a pH of 3.0 (beef extract buffer) or an elution buffer with high ionic strength (NaCl-HEPES buffer) can be applied.

MAF Disks are available with different functional groups attached to the surface. Depending on the sample and the preferred working conditions, the right MAF has to be chosen. Table 1 shows the available MAFs. Strong ion exchange groups are charged independently of the surrounding pH conditions. Weak ion exchange groups can lose their charge under very high or very low pH values. This can be favorable for elution.

Table 1: Available MAFs

| Name | Surface Chemistry | functional group | Charge |
|---|---|---|---|
| MAF-Q |  | Quaternary amine,<br>strong anion exchange | positive |
| MAF-DEAE |  | Diethylaminoethane,<br>weak anion exchange | positive |
| MAF-S |  | Sulfate,<br>strong cation exchange | negative |
| MAF-OH |  | Hydroxyl,<br>weak cation exchange | negative |

## Material

*Chemicals:*

- Glycine puriss p.a. (Sigma-Aldrich, Taufkirchen, DE, 33226-250G )
- Beef extract (Fluka, B4888-50G)
- Sodium hypochloride (Carl-Roth, Karlsruhe, 9062.3)
- HEPES (Sigma, H3375)

*Consumables:*

- Plastic Housing (Carl Roth, Karlsruhe DE, EH 30.1)

*Lab equipment:*

- PTFE holder, provided by TUM
- Fitting, provided by TUM
- Adapter (Carl Roth, Karlsruhe DE, EH 30.1)
- Pumpdrive 5206 Peristaltic Pump (Heidolph)

### *Buffers:*

**Elution Buffer** (NaCl-HEPES buffer):

- 0.05 M HEPES
- 1.5 M NaCl
- pH 7.0 for all MAFs

**Alternative Elution Buffer** (beef extract buffer):

- 0.5 M Glycine
- 3 % (w/v) Beef extract
- pH 9.5 for MAF-S and MAF-OH
- pH 3.0 for MAF-Q and MAF-DEAE

### *Centrifugal Ultra Filtration:*

- Amicon Ultra-15 Centrifugal Filters (Ultracel ® - 50 K; Regenerated Cellulose 50,000 MWCO
- Centrifuge (Universal 320R; Hettich Zentrifugen, Tuttlingen, Deutschland)

## Selection of MAF, buffer and pH conditions

Depending on the microorganisms that are to be concentrated, different conditions and buffers can be used. In most cases, samples should be concentrated at neutral pH conditions (about 7) and the NaCl-HEPES buffer at pH 7 should be used for the elution.

**NaCl-HEPES buffer:** NaCl-HEPES buffer uses ionic strength for the elution. Therefore, the pH value is adjusted to 7.0 for all kinds of MAFs.

**Beef extract buffer:** Beef extract buffer uses a change of pH for the elution. For negatively charged MAFs ( -OH and –S), the buffer is adjusted to pH 9.5. For positively charged MAF ( -DEAE and –Q) the buffer is adjusted to pH 3.0.

**Further tips for MAF-, buffer- and pH selection:**

If the monolithic column contains positively and negatively charged MAF-disks at the same time, samples should be filtrated at pH 7 and NaCl-HEPES buffer should be used for the elution at pH 7.0.

For concentration of Legionella, the samples should be adjusted to pH 3, MAF-OH should be used and beef extract buffer at pH 9.5 should be used for elution.

For concentration prior to PCR anaylsis, the NaCl-HEPES buffer is preferred, since beef extract inhibits the PCR. If beef extract buffer is used for samples that are subsequently analysed via PCR, a buffer exchange step is needed beforehand.

For samples that are to be analyzed via cell culture, NaCl-HEPES buffer can be used. If the concentrated microorganisms are sensitive to the high salt content of the buffer, eluted samples can be diluted with ultrapure water prior to cell culture.

For analysis via colilert test, both buffers are suitable.

## Construction of the Monolithic Column

To ensure the housing (1) will withstand the pressure during the filtration, the tip of the housing is cut off at ca. 0.9 cm length. To construct the monolithic column, the PTFE holder (2) is put into the housing. Afterwards an O-Ring (3) and the first monolithic disk (4) are put on top. If the capacity of one disk isn't sufficient or the sample is likely to block the monolith, it is possible to put a second O-ring (5) and monolithic disk (6) on top of the first one. Usually one monolithic disk should suffice. O-rings are needed to ensure the sample won't bypass the monolith.

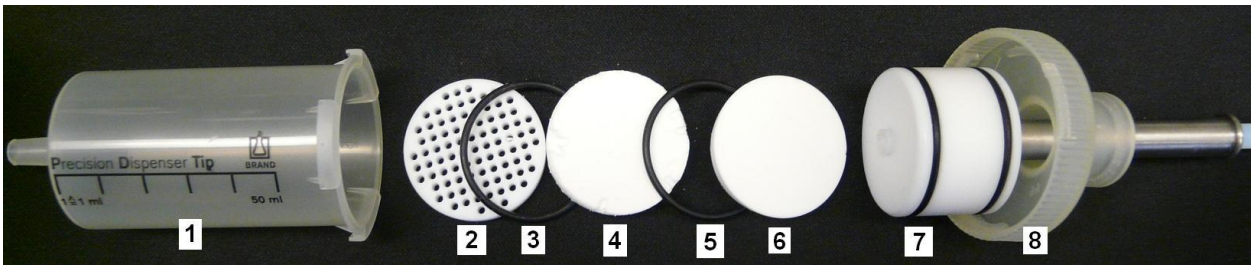



**Figure 1: BigMAF column consisting of 1: Housing, 2: PTFE holder, 3: O-Ring, 4: Monolithic disk, 5: O-Ring(optional), 6: Monolithic Disk (optional), 7: Fitting, 8: Adapter**

After the disks are inserted, the fitting (7) and adapter (8) are connected to the pump by a PTFE tube (Fig. 2 and 3). The pump is connected to a bottle with tap water. The pump is started at a low flow rate (ca. 200 mL/min) and the adapter is hold on top of the housing, until the housing is filled with water (Fig 3).

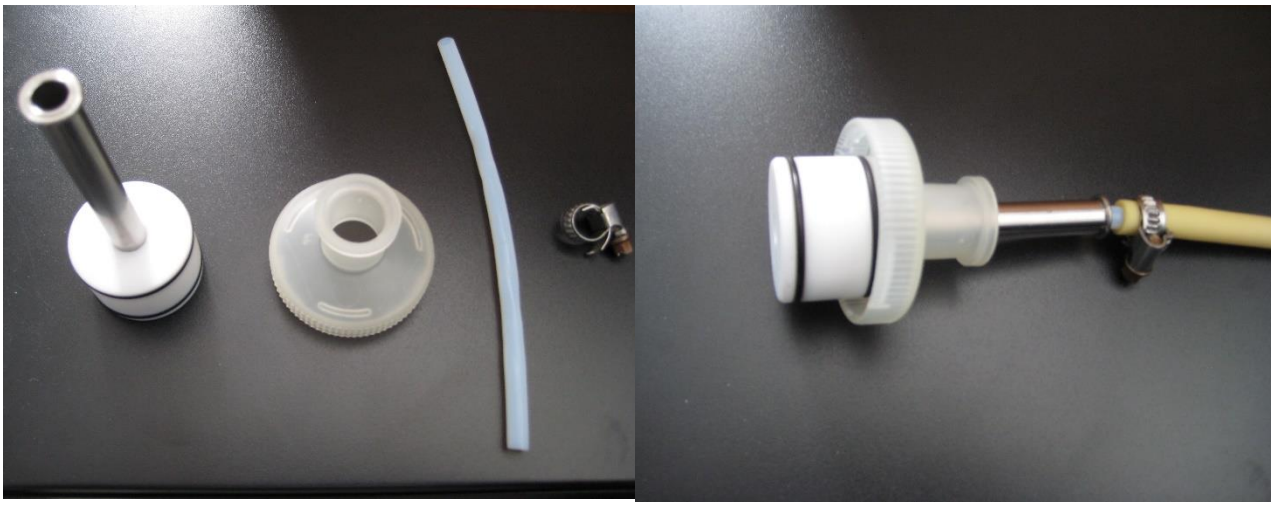


Figure 2: Connection of adapter and pump

Figure 3: Filling the column with water

After the housing is filled, the adapter is pushed into the housing, forcing the water inside through the MAF disk. It is important to leave no air bubbles inside the housing. Afterwards the adapter is locked with the fitting. Afterwards the flow rate is increased to 1 L/min and 1 L of tap water is pumped through the column to equilibrate it. After 1 L has passed through the column the pump is stopped and connected to the sample. The column has to be filled with water all the time, and no air bubbles should be put inside. The system is now ready for the filtration.

## Filtration

For the filtration process the column is fixated over a waste bag or bottle. The pump is connected to the sample (Fig. 4). A flow rate of 0.5 - 1 L/min is used. The diameter of the disks is optimized for the compression and pressure produced at this flow rate therefore it should not be varied. The column may not run dry during the filtration. After the filtration the column is emptied. Pumping air through the column after the filtration does not affect the elution. The rubber tube is disconnected from the fitting and the outlet of the housing is blocked.

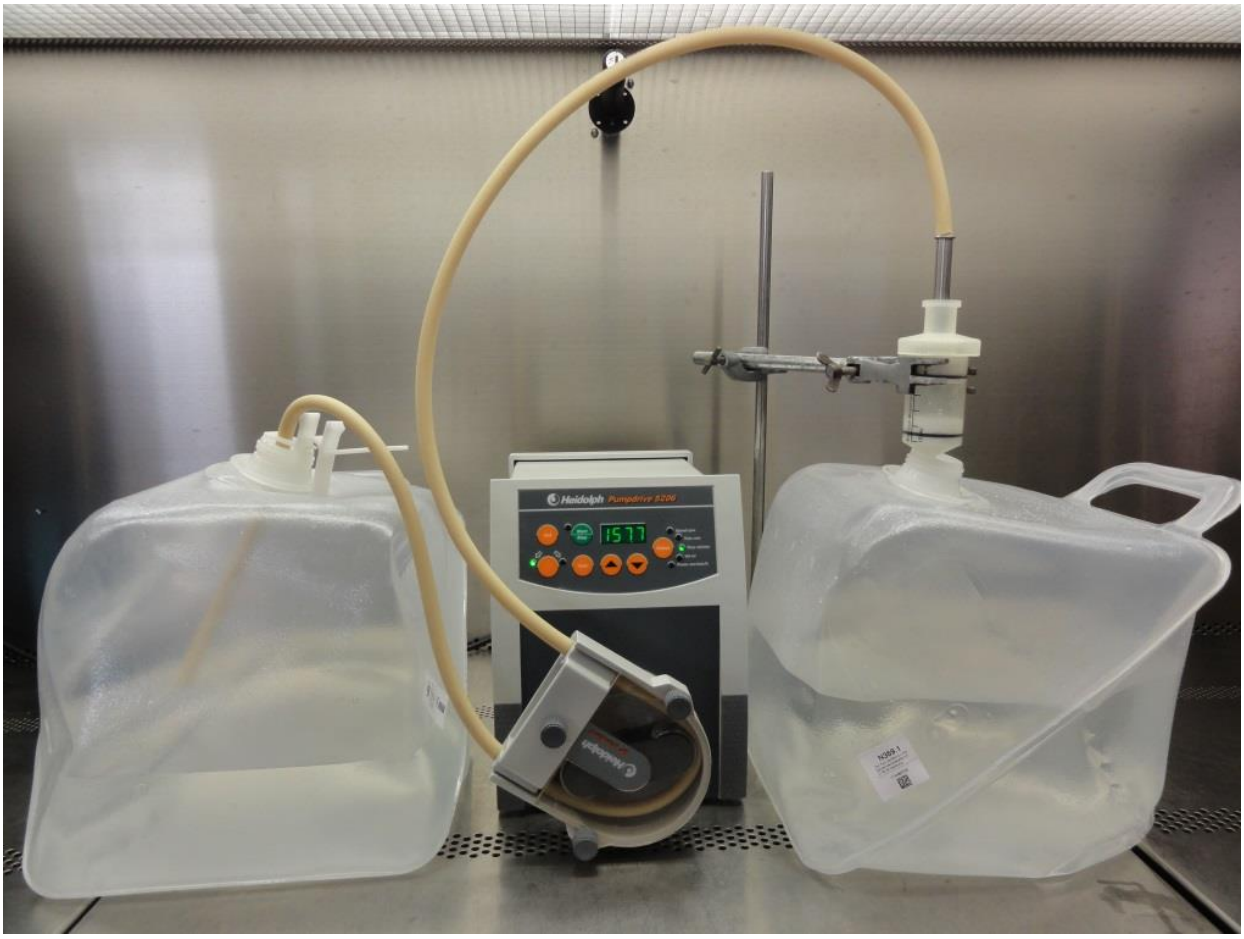



Figure 4: Sample bag (left), peristaltic pump (middle), filtrate bag and MAF column (right)

## Elution

If more than one monolithic disk was used in the column, each disk is eluted separately in a separate housing. For the elution, the rubber tube from the pump is disconnected from the fitting, the outlet is connected to a small piece of rubber tube that is blocked with a hose clamp or plug. 20 mL elution buffer are put into the housing with a syringe and a long needle (Fig. 5 and 6). Afterwards the rubber tube is reconnected to the fitting and the outlet is opened. The flow rate of the pump is set to 150 mL/min for the elution. After one third of the buffer has passed the column, the pump is switched off, the outlet of the housing is blocked with a hose clamp again and the disk is incubated for 2 minutes. Afterwards the outlet is opened again and another third of the buffer is pumped through the column. The outlet is closed again and the disk is incubated for another 2 minutes. Afterwards the last third of the buffer passes the column. For the analysis, samples can be adjusted to pH 7.

Note: Monolithic disks and housing are disposable parts and should not be used for more than one sample, in order to prevent cross contamination.

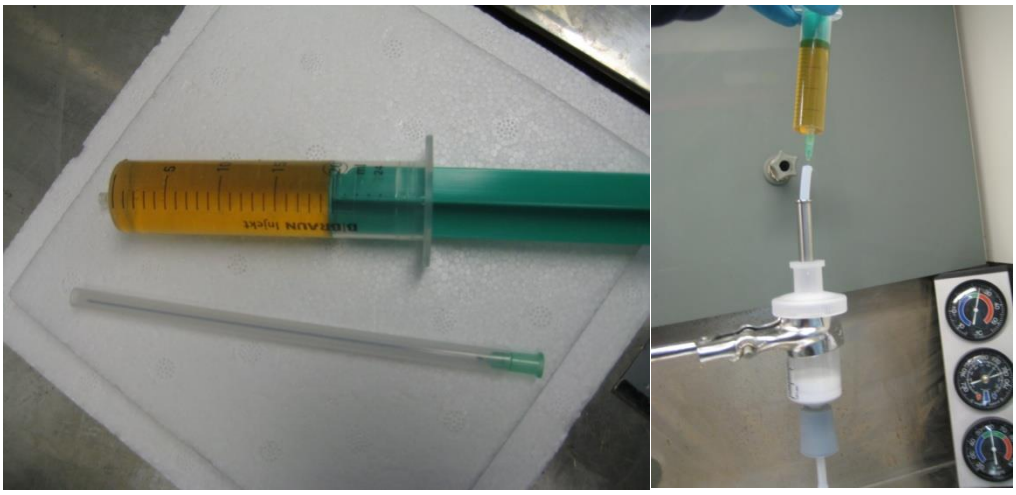


Figure 5: Injection of elution buffer

Figure 6: MAF column filled with elution buffer, outlet is closed with a plugged rubber tube

## Cleaning

After the filtration the fitting, adapter and PTFE holder are cleaned with 70% ethanol and Milli Q water. The rubber tube is cleaned with sodium hypochloride and afterwards Milli Q water. The disposable parts (housing, monolithic disks) are disinfected by autoclave. Waste bottles are autoclaved as well.

## Further Concentration

For further concentration from 20 mL to 1 mL, Amicon ultra centrifuge filters can be used. Filters are prewetted with 20 mL ultrapure water, followed by centrifugation (1900xg for 8 min). Filters may not run dry after moistening.

Centrifugation:

Spin rate: 1900 × g, time depends on the volume of the sample. For a concentration from 15 mL to 0.5 mL a centrifugation time of 5 min is sufficient. The final concentrate is eluted using a 200 µL

pipette in a side-side sweeping motion or by vortexing the whole tube and then picking up the sample with the 200 µL pipette.

## References

1. Peskoller, C., R. Niessner, and M. Seidel, *Development of an epoxy-based monolith used for the affinity capturing of Eschericha coli bacteria.* Journal of Chromatography A, 2009. **1216**(18): p. 3794-3801.