Biotage PhyTip® 5K and 30K Desalting Columns

This specification sheet provides details on PhyTip 5K and 30K desalting columns.

PhyTip desalting columns are unique desalting tools from Biotage designed for high throughput micro volume desalting of purified protein samples. Patents pending PhyTip desalting columns are available for use on the MEA 2 System and are compatible with popular liquid handling robots found in the laboratory.

PhyTip desalting columns are available in the 1000+ column size; each column contains 200 µL or 600 µL of 5K or 30K gel filtration resin and supports a recommended sample volume range of 20 to 400 µL.

Shipping and Storage

Shipping Solvent

Note: PhyTip desalting columns were previously shipped in sterile water and did not require pre-conditioning. PhyTip columns are now shipped in preservative to improve shelf-life and now require pre-conditioning.

Each pack of PhyTip desalting columns has been manufactured and QC'd to the highest standards and shipped in retainer boxes that have been shrink-wrapped to maintain the integrity of the specific resin within each PhyTip desalting column. The shipping buffer 0.1% calcium hypochlorite and is nontoxic. Please see the SDS document for more details.

Storage

- This product is shipped at ambient temperatures, but on receipt should be stored in a standard laboratory refrigerator between 4 and 8 °C.
- Do NOT freeze or store frozen.
- During long term storage, maintain 500 mL of water (or 0.1% calcium hypochlorite) to the box of columns and keep the lid of the box closed; store in the refrigerator.
- Use columns within 1 year of receipt.

Conditioning Procedure

Conditioning is recommended to remove the 0.01% calcium hypochlorite solution prior to use. This is accomplished by passing at least 3 bed volumes and up to 4 bed volumes of working DI water or final buffer through the column immediately prior to use.

The liquid handler conditioning time may be reduced with the following preconditioning overnight procedures:

Day 1 (overnight conditioning step):

1. Remove the columns from the box.
2. Fill the container with final buffer you want to exchange into.
3. Place the columns back in the box that contains buffer.
4. The columns are conditioned by diffusion overnight.

Day 2 (desalting/buffer exchange):

1. Take columns out of buffer and let the fluid drip out (fluid flow will stop at the top frit.)
2. Add 2 x 600 µL of final buffer (for 200 µL resin bed add 2x 300 µL) above the resin bed. This can be performed by the liquid handler.
3. When the fluid meniscus reaches the top frit, the flow will stop.
4. The columns are ready for sample addition.

Use of PhyTip Desalting Columns and MEA Personal Purification System

Using the MEA 2 Personal Purification System, place the following plates and boxes in the appropriate positions. Reach out to a Biotage representative for use on other robotic instruments:

Conditioning Buffer

The conditioning step allows the PhyTip columns to exchange from shipping liquid to the final buffer condition. Refer to the protocol above for proper conditioning. Recommended final buffers and for conditioning include low-salt, protein buffer for desalting applications, and PBS (Phosphate Buffer with NaCl, pH 7.4) for buffer exchange. Other buffers can be used.

Sample

Load the sample to the top of the PhyTip column and collect flow through. Refer to Table 1 for suggested load volumes. The recommended sample volume that is transferred to the PhyTip columns is 20–400 µL. Ensure that no air bubbles are transferred along with the sample. Air bubbles transferred to the PhyTip columns may prevent the flow of liquid over the size exclusion medium which can result in a reduced recovery of sample volume and a reduction in mass of desalted protein.

Elution Buffer

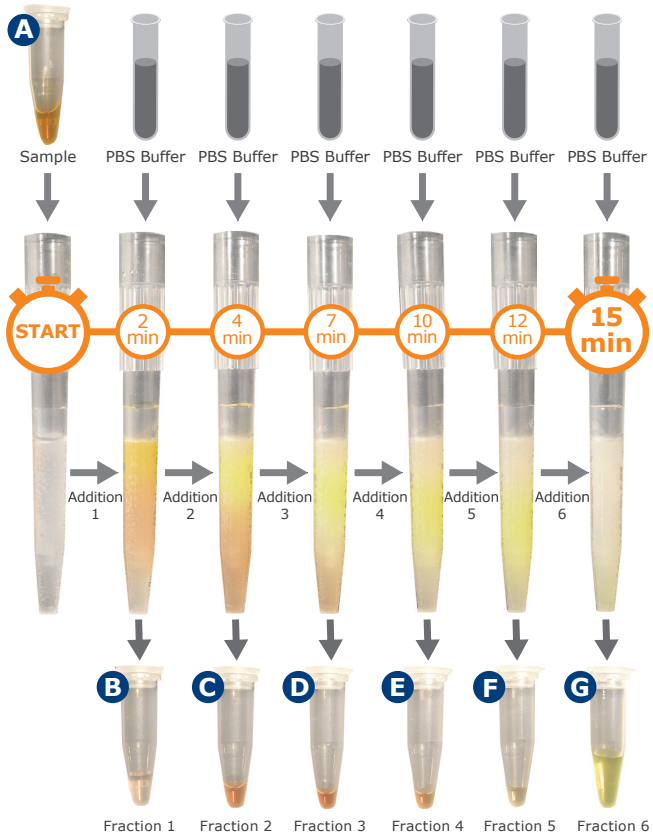
Elution Buffer is used in the release of the target molecule from the PhyTip column medium. The volume of elution buffer should maximize sample recovery through the desalting column and maximize the removal of unwanted salts. Refer to Table 1 for suggested elution volumes. Elution buffers should be the same buffer as the Conditioning Buffer and include PBS (Phosphate Buffer with NaCl, pH 7.4) or other suitable protein storage buffers.

Sample and Elution Volume Guidelines

Each PhyTip column requires an elution buffer to recover the target molecule. The elution volume used is specific for the sample volume. The following guidelines are optimized for a high salt removal. For increased protein recovery, elution volumes may be increased.

Table 1. Suggested Sample and Chaser Volumes for Phytip Desalting Columns

Column Bed Size (µL)	Sample Vol. (µL)	Recommended Elution Vol. (µL)
200	20	150
200	30	140
200	40	130
200	50	120
200	60	110
200	70	100
200	80	90
600	100	400
600	200	300
600	300	200
600	350	150



(Top) Microfuge tube Sample A containing brown myoglobin protein (16.7kDa) and yellow DNP-glutamate salt (313Da) was loaded onto a 600 µL PhyTip desalting column.
The same PhyTip® column is shown at different steps of desalting.
From left:
START Column conditioned by PBS buffer prior to sample loading
2 min Column after 200 µL sample has entered the resin bed
4 min-12 min Column after 100 µL PBS buffer is applied
15 min Column after final elution volume of 400 µL PBS buffer added

Microfuge tubes from left:
A 200 µL starting sample
B Flow through collected after sample is applied
C-F Fractions collected after each 100 µL PBS buffer
G Fraction collected after final 400 µL PBS buffer

Ordering Information

For Ordering informtion please visit: www.biotage.com

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