User Guide

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AdvanceBio Spin Columns

Analytical and semipreparative columns and 96-sample plates for desalting or buffer exchange of biomolecules

Introduction

Agilent AdvanceBio Spin columns and 96-sample plates are designed for efficient sample preparation of proteins or oligonucleotides under aqueous conditions, preserving their native structure with suitable ionic strength and pH. Excess salt or other incompatible small molecule matrix components may interfere with sample analysis, leading to poor, confusing, or inaccurate results. These products are filled with bead-based, cross-linked dextran that has either been autoclaved or has a preservative included in the shipping solution. A centrifuge-based approach minimizes the time required for data-quality-enhancing sample preparation.

Chemical compatibility

The gel filtration medium is compatible with all commonly used buffers, as well as solutions containing up to 0.2 M NaOH, 0.2 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine HCl, 1% SDS, 24% ethanol, 30% propanol, or 30% acetonitrile. It is stable between pH 2.0 and 13.0.

Required equipment and materials

For the AdvanceBio Spin column, < 100 µL (part number 1980-1103):

- Centrifuge capable of accommodating 1.5 mL sample tubes

For the AdvanceBio Spin 96-sample plate (part number 1980-1104):

- Centrifuge capable of accommodating stacked plates (5.1 cm height)
- Re-usable 96-well wash plate (part number 5043-9308 or similar with at least 400 µL capacity/well)
- 96-Well collection plate (part number 5043-9312 or similar)
- Extra adapters (optional, part number 1980-1106)
- Sealing film (optional, part number 5042-1389 or similar)
- Multichannel pipettor and tips

For the AdvanceBio Spin column, < 1,000 µL (part number 1980-1105):

- 50 mL Centrifuge tubes for wash and sample collection (two per column; part number 5610-2049, 190065200, or similar)
- Centrifuge capable of accommodating 50 mL tubes

Using your spin columns

		Agilent AdvanceBio Spin Columns Samples < 100 µL	Agilent AdvanceBio Spin 96-Sample Plate Samples < 50 µL	Agilent AdvanceBio Spin Columns Samples < 1,000 µL
Storage Solution		Autoclaved water	Water with ProClin 150	Water with ProClin 150
Storage Temperature		Room temperature; **do not freeze**		
MWCO		For biomolecules: > 5 kDa (proteins), > 10 nt (oligonucleotides)		
Recommended Sample Volume		20 to 100 µL (Optimized for 50 µL. For sample volumes < 20 µL, dilution to increase the volume to 20 µL is recommended.)	10 to 50 µL	1,000 µL (For low sample volumes, one may need to dilute the sample to increase the volume and ensure good levels of recovery)
Centrifuge Conditions		1,000 g for 2.0 minutes; swinging bucket or fixed rotor*	1,000 g for 3.0 minutes; swinging bucket	800 g for 2.5 minutes; swinging bucket (May also be used under gravity conditions. Recovery, efficiency, and dilution may differ)
		To provide the correct force, it is essential that the centrifuge be set up correctly for the rotor being used. It is also important that the rotor is balanced appropriately, according to the manufacturer's instructions.		
Collection Tube/Plate		1.5 mL Microcentrifuge tube (supplied)	Wash step: 96-well plate Collection step: 96-well plate	50 mL centrifuge tubes
Conditioning and Equilibration	1	Snap or twist to remove the outlet sealing cap. Place each column in a wash tube. Remove the inlet screw cap and place the assembly into the rotor.	Remove the outlet sealing film and place the AdvanceBio Spin 96-sample plate onto the wash plate. Remove the inlet sealing film and place the assembly into the swinging rotor.	Remove the inlet sealing cap from the column and pour out any residual storage buffer. Place the column into an adapter, remove the outlet sealing cap, and place the column with the adapter into a 50 mL centrifuge tube. Apply 1 mL of water or buffer of your choice to each column.**
	2	Spin.	Spin.	Spin.
	3	Discard the storage buffer from the wash tube.	Discard the storage buffer from the wash plate.	Discard the storage buffer from the wash tube.
	4	Slowly apply 400 µL of water or buffer of your choice to the center of each column, taking care not to disturb the surface of the stationary phase.**	Slowly apply 400 µL of water or buffer of your choice to the center of each well, taking care not to disturb the surface of the stationary phase.**	Apply 1 mL of water or buffer of your choice to each column.**
	5	Spin.	Spin.	Spin.
	6	Discard the wash buffer from the wash tube and repeat steps 4 to 6 two to three times.	Discard the wash buffer from the wash plate and repeat steps 4 to 6 two to three times.	Discard the wash buffer from the wash tube and repeat steps 4 to 6 one to two times.
		The matrix in the column or wells should appear opaque. If it is translucent or shiny, the centrifugation conditions were not correct. Verify that the centrifuge speed is correct for the rotor radius. Timing should begin after the set speed has been reached. An external timer may be necessary.		

		Agilent AdvanceBio Spin Columns Samples < 100 µL	Agilent AdvanceBio Spin 96-Sample Plate Samples < 50 µL	Agilent AdvanceBio Spin Columns Samples < 1,000 µL
Sample Application	1	Replace the wash tube with a clean collection tube.	Replace the wash plate with a clean collection plate.	Replace the wash tube with a clean collection tube.
	2	Using a pipette, slowly apply sample to the center of each tube, taking care not to disturb the surface of the stationary phase.	Using a pipette, slowly apply sample to the center of each well, taking care not to disturb the surface of the stationary phase.	Using a pipette, slowly apply sample to the center of each tube.
	3	Spin.	Spin.	Spin.
	4	Discard the used AdvanceBio Spin column.	Discard the used AdvanceBio Spin 96-sample plate.	Discard the used AdvanceBio Spin column.
	5	Fit a cap to the collection tubes for storage or use immediately (to prevent evaporation).	Use a sealing mat on the collection plate for storage or use the samples immediately (to prevent evaporation).	Fit a cap to the collection tubes for storage or use immediately (to prevent evaporation).

* When using a fixed rotor, the stationary phase bed will become angled. Ensure that the cartridge is placed into the rotor in the same orientation each time.

** If the storage solution matches the buffer of your choice, skip to the sample application, as there is no need to equilibrate the gel filtration bed with another buffer.

Note: Some biomolecules may precipitate in pure water or with low ionic strength.

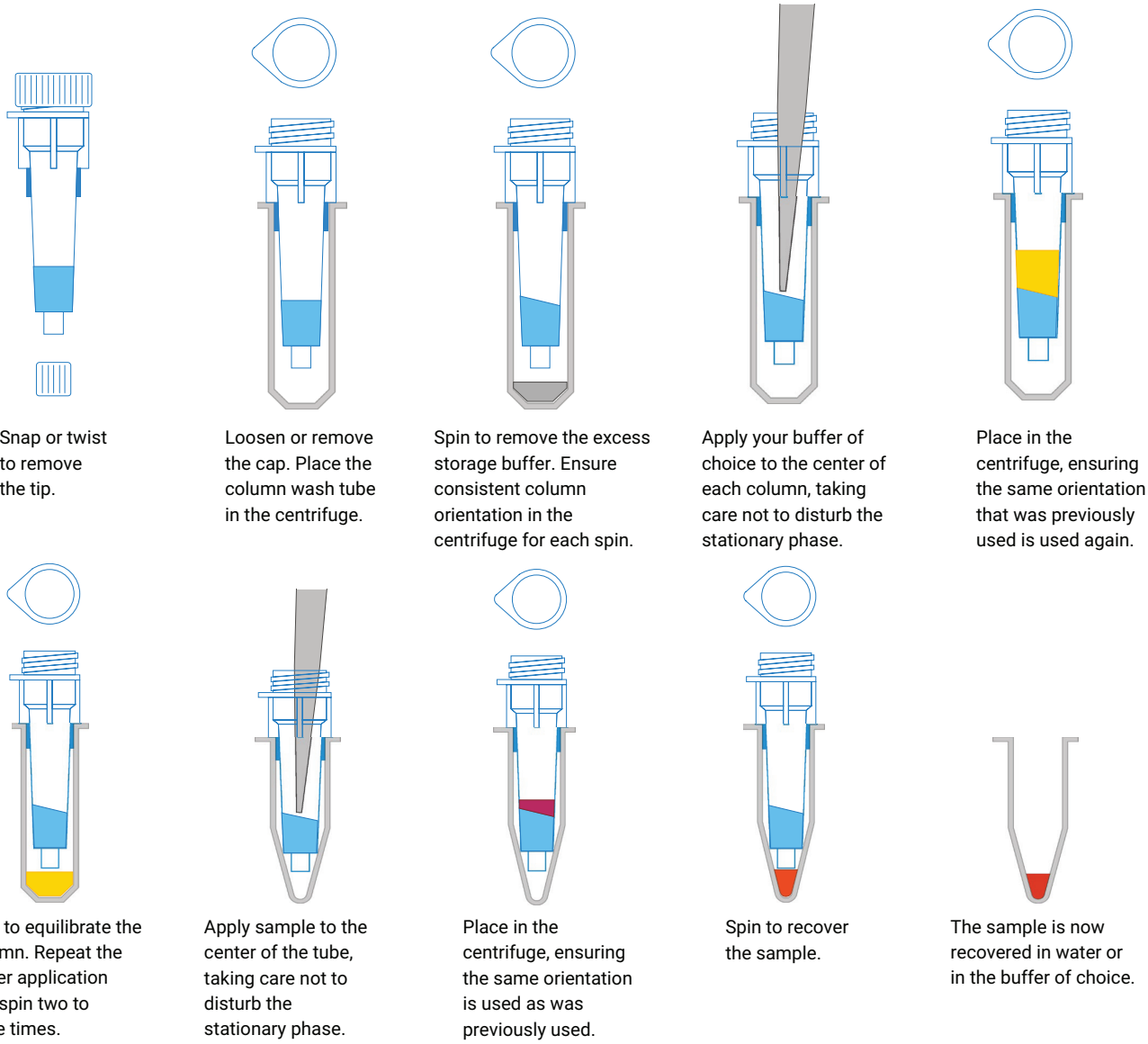

Figure 1. Step-by-step illustrated guide for the use of AdvanceBio Spin columns.

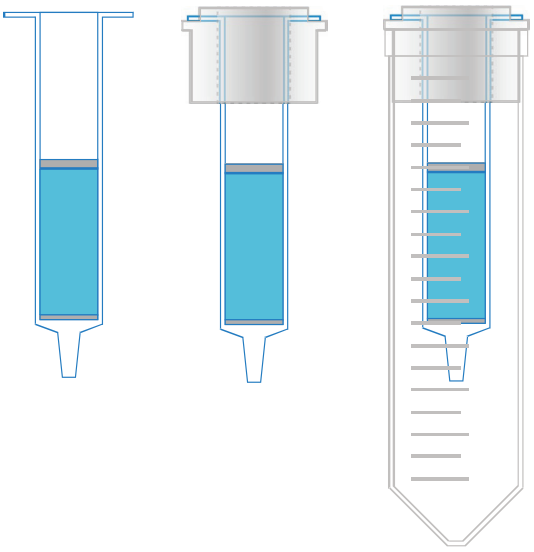

Figure 2. Assembly of part number 1980-1105 with re-usable adapter and 50 mL centrifuge tube.

Ordering details

Description	Part Number
AdvanceBio Spin Columns for Desalting or Buffer Exchange, < 100 µL Samples, 25/pk, Collection Tubes Included	1980-1103
AdvanceBio Spin 96-Sample Plate for Desalting or Buffer Exchange, 10 to 50 µL Samples, 1/pk	1980-1104
AdvanceBio Spin Columns for Desalting or Buffer Exchange, < 1,000 µL Samples, 50/pk Columns, Plus 4 Re-Usable Adapters	1980-1105
AdvanceBio Spin Column Re-Usable Adapters, 8/pk (For Optional Use with p/n 1980-1105)	1980-1106
96-Well Plate, Polypropylene, 1.2 mL, 27 mm, Round Wells, U Shape, 25/pk (Recommended for Wash Steps with p/n 1980-1104)	5043-9308
96-Well Plate, Polypropylene, 0.33 mL, 14 mm, Round Wells, V Shape, 25/pk (Recommended for Final Collection Step with p/n 1980-1104)	5043-9312
Sealing Mat, 96 wells, Round, Preslitted, Silicone, 50/pk	5042-1389
Centrifuge Tube, Polypropylene, Graduated, 29 mm od, 115 mm, 50 mL, Conical Base, Wide Neck, Threaded Top, 25/pk	5610-2049
Centrifuge Tube, Polypropylene, Graduated, 29 mm od, 115 mm, 50 mL, Skirted Conical Base, Wide Neck, Threaded Top, 500/pk	190065200

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