CYTODIAGNOSTICS

PRODUCT DATA SHEET

MWCO Columns

Description

Centrifugal filters are designed as disposable, single-use devices specifically for ultrafiltration. These filters utilize polyethersulfone (PES) membranes, making them ideal for the centrifugal concentration and purification of biological samples.

The design ensures that biological macromolecules can be efficiently concentrated or purified without denaturing them, maintaining their integrity and functionality.

Storage

Store centrifugal filters at room temperature.

Product Safety and Handling

This product is for R&D use only. Please review the safety datasheet (SDS) available online for proper safety and handling procedure.

Major Uses for Ultrafiltration

- Concentration of antibodies, oligonucleotides, proteins, enzymes, cells, and biomolecules.
- Removal of salts, non-aqueous solvents, and low molecular weight materials.
- Removal of labeled amino acids and nucleotides, HPLC sample preparation, deproteinization of samples, and recovery of biomolecules from cell culture supernatants and lysates.
- Purification of gold conjugates **For 100 MWCO membranes **

Choice of Centrifugal Filters

- CytoColumn™ 0.5mL Centrifugal Filters: For 0.1 to 0.5 mL samples.
- CytoColumn™ 5mL Centrifugal Filters: For 2 to 4 mL samples.
- CytoColumn™ 15mL Centrifugal Filters: For 5 to 15 mL samples.

Choice of Molecular Weight Cut-off Membranes

For optimal recovery, select a membrane with a MWCO at least half the molecular weight of the solute to be retained. This ensures efficient retention and minimizes target molecule loss.

CytoColumn™ products, featuring advanced design and low adsorption materials, provide faster processing and higher sample recovery, typically exceeding 80% recovery for samples with over 0.1 mg/mL solute concentration.

Proper selection of device size and membrane cut-off is important for achieving high recovery rates while minimizing nonspecific binding to the membrane and container surfaces.

Adsorption to the Membrane

The effectiveness of centrifugal filters can be influenced by the adsorption of solutes to the membrane surface.

- Solute adsorption on the membrane surface is typically between 2 to 10 $\mu g/cm^2$. This can increase to 20 to 100 $\mu g/cm^2$ when the filtrate passes through the entire internal structure of the membrane.
- Higher MWCO membranes tend to bind more solute compared to lower MWCO membranes. Therefore, select the appropriate MWCO to minimize unwanted adsorption.
- The choice of low adsorption materials helps reduce solute binding. However, some adsorption to the membrane and the internal surface of the sample container is inevitable.

Proper selection and handling of membranes can minimize adsorption and improve recovery.

General Operational Protocol

1. Choose a membrane cut-off (MWCO) that is at least 50% smaller than the molecular weight of the species you intend to concentrate. This helps maximize recovery.
2. Fill the centrifugal filters with up to the maximum volumes specified for each filter type. Ensure the screw closure is fully seated.
3. Place the assembled centrifugal filters into the centrifuge. For fixed angle rotors, ensure the printed window faces upwards/outwards.
4. Centrifuge the filters at the speeds recommended for the specific membrane type and MWCO. Do not exceed the maximum g force indicated.
5. Once the desired concentration is achieved, remove the assembly and use a pipette to recover the sample from the bottom of the concentrate pocket.

Maximum Centrifugal Force (x g)

- 0.5 mL Units: 10,000 x g
- 4 mL Units: 4,000 x g
- 15 mL Units: 3,000 x g

Desalting and Purification Operation

1. Concentrate the sample to the desired level.

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2. Empty the filtrate container.
3. Refill the centrifugal filters with an appropriate water or desired buffer.
4. Concentrate the sample again. Typically, three wash cycles will remove 99% of the initial salt content.

Purification of Nanoparticle Conjugates Using CytoColumn™

1. **IMPORTANT:** If your product or any downstream applications are sensitive to glycerine, make sure to rinse the filtration device with ddH_2O or buffer before use. Trace amounts of glycerine are present in the filtration membrane to prevent drying out.

2. Transfer your conjugated sample into the appropriate CytoColumn™ (see Page 4).

   Note I. Ensure that the molecular weight cut-off (MWCO) of the CytoColumn™ is suitable for the components being filtered out (i.e., the reactants being removed should have a lower molecular weight than the cut-off of the column). The recommended MWCO is 100 kDa for nanoparticle products.

   Note II. Do not overfill the CytoColumn™, such that there is still some space left. This will mitigate any leakage between the two column components during centrifugation.

3. Using a suitable centrifuge, centrifuge the columns according to the table below, making sure to always use a counterbalance. If there is more volume than the filter device can hold, the remainder of the sample or any wash solutions can be poured into the unit on top of the purified product and centrifuged again. Make sure to always empty contents collected at the bottom of the tube between each centrifugation.

   Table 1. *Recommended centrifugation speeds and times for different volume CytoColumn™.*

Column Size	Centrifugation Speed (x g)	Centrifugation Time
0.5 mL	10,000	10 min.
4 mL	1,700	10 min.
15 mL	1,700	10 min.

   Note. Centrifugation times will vary based on the MWCO, with smaller MWCO devices requiring longer centrifugation. If the remaining volume of purified product is more than desired, subsequent centrifugations can be done.

4. Following centrifugation, carefully collect the purified product using a micropipette. A small volume of collection buffer can be used to rinse and collect any leftover product on the membrane.

   Note: The CytoColumn™ can be re-used but ensure that the membrane does not dry out between uses. In the event of drying out, the CytoColumn™ is no longer useable.

5. The purified product is now ready for analysis and any subsequent downstream applications.

Purification of Protein/Oligonucleotide Solutions and Buffer Exchange Using CytoColumn™

1. **IMPORTANT:** If your product or any downstream applications are sensitive to glycerine, make sure to rinse the filtration device with ddH_2O or buffer before use. Trace amounts of glycerine are present in the filtration membrane to prevent drying out.
2. Transfer your protein or oligonucleotide solution into the appropriate CytoColumn™ (see Page 4).

   Note I: The molecular weight cut-off (MWCO) of the CytoColumn™ should be lower than the molecular weight of the protein/oligonucleotide being purified. This ensures the desired product is caught, while wash buffers and undesired reactants pass through.

   Note II: If the undesired reactants have a greater molecular weight than your product, then the MWCO of the column should be larger than the desired product and smaller than the unwanted reactants. The desired product will thus be found in the filtrate following centrifugation.

   Note III: Do not overfill the CytoColumn™, such that there is still some space left. This will mitigate any leakage between the two column components during centrifugation.

3. Using a suitable centrifuge, centrifuge the columns according to the table below, making sure to always use a counterbalance. If there is more volume than the filter device can hold, the remainder of the sample or any wash solutions can be poured into the unit on top of the purified product and centrifuged again. Make sure to always empty contents collected at the bottom of the tube between each centrifugation.

   Table 2. *Recommended centrifugation speeds and times for different volume CytoColumn™.*

Column Size	Centrifugation Speed (x g)	Centrifugation Time
0.5 mL	10,000	10 min.
4 mL	1,700	10 min.
15 mL	1,700	10 min.

   Note. Centrifugation times will vary based on the MWCO, with smaller MWCO devices requiring longer centrifugation. If the remaining volume of purified product is more than desired, subsequent centrifugations can be done.

4. Following centrifugation, carefully collect the purified product using a micropipette. A small volume of collection buffer can be used to rinse and collect any leftover product on the membrane.

   Note: The CytoColumn™ can be re-used but ensure that the membrane does not dry out between uses. In the event of drying out, the CytoColumn™ is no longer useable.

5. The purified product is now ready for analysis and any subsequent downstream applications.

Troubleshooting:

Flow Rate Issue

The flow rate through centrifugal filters is influenced by several factors: MWCO and porosity, sample concentration, viscosity, centrifugal force, and temperature.

Lower MWCO membranes and higher sample concentrations increase viscosity, leading to slower flow rates. Viscous solutions, like those with 50% glycerin, can take up to five times longer to concentrate than buffer solutions.

Higher centrifugal force increases the flow rate but should not exceed recommended levels to avoid membrane damage.

Additionally, operating at 4°C results in flow rates approximately 1.5 times slower than at 25°C. Adjusting these factors optimizes the flow rate for efficient sample processing.

Prerinsing

Centrifugal filter membranes contain trace amounts of glycerin and sodium azide, which may interfere with analyses.

To remove these, rinse the filter with a buffer solution or deionized water and centrifuge it. Decant the filtrate afterward.

If not used immediately, store the prerinsed device in the refrigerator with buffer or water covering the membrane to prevent it from drying out.

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Table 1: Recovery for 0.5 mL Column (Spin Condition at 10,000g, room temperature, 0.5 mL starting volume, n=6)

Protein	MWCO (PES)	Retention
Cytochrome C	5,000	>95%
BSA	10,000	>95%
BSA	30,000	>95%
IgG	50,000	>80%
IgG	100,000	>90%

Table 2: Recovery for 5 mL Column (Spin Condition at 4,000g, room temperature, 5 mL starting volume, n=4)

Protein	MWCO (PES)	Retention
Cytochrome C	5,000	>90%
BSA	10,000	>95%
BSA	30,000	>95%
IgG	50,000	>80%
IgG	100,000	>80%

Table 3: Recovery for 15 mL Column (Spin Condition at 3,000g, room temperature, 15 mL starting volume, n=2)

Protein	MWCO (PES)	Retention
Cytochrome C	5,000	>80%
BSA	10,000	>95%
BSA	30,000	>95%
IgG	50,000	>80%
IgG	100,000	>90%

Catalog Number	Description	Sizes
MWC-3-X*-Y*	MWCO Ultrafiltration Spin Columns, 3 kDa	0.5ml, 4ml, 15ml
MWC-10-X*-Y*	MWCO Ultrafiltration Spin Columns, 10 kDa	0.5ml, 4ml, 15ml
MWC-30-X*-Y*	MWCO Ultrafiltration Spin Columns, 30 kDa	0.5ml, 4ml, 15ml
MWC-100-X*-Y*	MWCO Ultrafiltration Spin Columns, 100 kDa	0.5ml, 4ml, 15ml

X* Indicates column volume, 05 for 0.5ml, 4 for 4ml, 15 for 15ml
Y* Indicates number of columns, 1 for 1 column, 5 for 5 columns. i.e. MWC-3-05-1, 3kDa cut-off, 0.5ml column pkg size of 1

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