

# Whatman Ion Exchange Celluloses: DE52, CM52, DE32, and CM32

## Instructions

The range of Whatman™ cellulose based ion exchange resins has been developed specifically for efficient separation of biopolymers, e.g., proteins, enzymes and nucleic acid fragments.

### Resins range

Type	Anion exchange	Cation exchange
Dry	DE32	CM32
Preswollen	DE52	CM52

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Please read these instructions carefully before using the products.

## Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

# 1 BioProcess resins

BioProcess™ chromatography resins are developed and supported for production scale chromatography. All BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

## 2 Resins characteristics

**Table 1.** Resins characteristics.

Type	Anion exchange	Cation exchange	Physical form	Functional group	Ionic capacity (meq/dg) <sup>1</sup>
Dry	DE32		Micro-granular	Diethyl-aminoethyl	0.88 to 1.08
Dry		CM32	Micro-granular	Carboxy-methyl	0.90 to 1.15
Preswollen	DE52		Micro-granular	Diethyl-aminoethyl	0.88 to 1.08
Preswollen		CM52	Micro-granular	Carboxy-methyl	0.90 to 1.15

<sup>1</sup> meq/dg= milli equivalents/dry gram resin.

### Notes on general handling

#### Do

- Store the products at 4°C to 30°C. Avoid freezing and direct sunlight.

- Always keep product sealed when not in use.
- Always use distilled or preferably de-ionized water.

### **Do not**

- Do not subject the products to concentrated acids, alkalis or strong oxidizing agents for extended periods.

For the purposes of regeneration, sterilization and depyrogenation, the resins may be subjected to 0.5 to 2.0 M NaOH for up to 48 h.

- Do not macerate or stir slurries too vigorously in order to avoid the generation of fines.

## 3 Preparation of ion exchange celluloses in dry form

### **Introduction**

For dry ion exchange celluloses (DE32 and CM32), all stages of pretreatment must be carried out as described below in order to obtain the best possible performance.

### **Preparation**

#### **Precycling**

**Table 2.** Order of treatment.

Type	First treatment	Intermediate pH	Second treatment
DE	0.5 M HCl	4.0	0.5 M NaOH
CM	0.5 M NaOH	8.0	0.5 M HCl

Step	Action
1	Stir the weighed ion exchanger into 15 volumes (w/v) of "First treatment" solution (see Table above).
2	Leave for at least 30 min.
3	Filter or decant off supernatant and wash until the filtrate reaches the "Intermediate pH" (see Table above).
4	Stir the ion exchanger into 15 volumes of "Second treatment" solution and leave for further 30 min. Filter off supernatant.
5	Repeat second treatment (Step 4) followed by washing until the filtrate is near neutral.

## 4 Preparation of ion exchange celluloses in preswollen form and for dry forms after precycling

### Introduction

Preswollen ion exchange celluloses (DE52 and CM52) can be used without initial precycling but must be equilibrated completely.

**Note:** *Preswollen ion exchangers should never be dried, by any method, at any stage of pretreatment, or in subsequent use.*

The technique described below is a general method which can be applied to all preswollen ion exchange cellulose resins.

## Laboratory scale preparation

### Slurry preparation

Equilibration volumes required for all resins are less when using buffers in which the buffering ion is the co-ion, i.e., carries a charge of the same sign as the ion exchanger.

For example, when Tris-HCl buffers are used for DE products, the equilibration time and volume will be less than if used with acetate based buffers.

In all cases, it is advantageous to initially pre-equilibrate the ion exchanger with a buffer of substantially higher concentration (typically ten-fold) than the required initial starting buffer concentration. This is the basis for the *Preferred method* described below.

After column packing the ion exchanger must be equilibrated with the starting buffer before sample application. This is normally achieved by passing 2 to 4 bed volumes of the low concentration buffer through the packed column.

### Preferred method

Step	Action
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- |   |   |
|---|---|
| 1 | Gently stir the ion exchanger with a concentrated solution (0.2 to 1.0 M) of the buffer to be used in the initial separation step for 2 to 3 min. About 15 to 30 mL of buffer are used for every dry gram of resin initially taken, or about 6 mL/g of wet ion exchanger. |
| 2 | Whilst stirring, adjust the pH of the buffer/ion exchanger slurry to the desired pH value with the acidic or basic component of the buffer.   |

Step	Action
3	Allow the slurry to settle and decant the supernatant containing any fines.
4	Re-disperse the ion exchanger in the buffer. The total volume of the slurry should be 30 mL/g of dry ion exchanger used or about 6 mL/g of wet ion exchanger.
5	<p>Allow the slurry to settle in a suitable measuring cylinder in an area free of draughts, direct sunlight, heaters etc.</p> <p>The time allowed for settling is calculated from <math>t = n \times h</math> where <math>t</math> = time (min), <math>h</math> = the total height of suspension in the measuring cylinder (cm), and <math>n</math> = a factor between 1.3 and 2.4.<sup>1, 2</sup></p>
6	<p>a. Note the "wet settled volume". This is the volume occupied by the ion exchanger after settling under prescribed conditions.</p> <p>b. Immediately remove the supernatant containing fines so that the final volume remaining in the measuring cylinder is the "wet settled volume" plus 20%.</p>

<sup>1</sup> The choice of a suitable value of  $n$  depends on the degree of fines removal required. The lower the value of  $n$  the shorter the settling time and the more material removed/discarded. It should not be necessary to use a value of  $n$  less than 1.3 to achieve the desired flow characteristics, and to do so would result in indiscriminate loss of material. When  $n = 2.4$ , only the finest partials will be removed. The dry products DE32 and CM32 are more susceptible to fines generation (during manufacture) than the wet DE52 and CM52.

<sup>2</sup> To achieve the optimal flow rate, it may be necessary to use  $n = 1.3$  for DE32 and CM32 whereas with DE52 and CM52  $n = 2.4$  will be adequate.

## Alternative method – Aliquot buffer changes

Step	Action
------	--------

- |   |   |
|---|---|
| 1 | Gently stir the ion exchanger into a volume of the buffer as in Step 1 of the <i>Preferred method</i> above.  |
| 2 | Leave for 10 min and decant off, or filter the supernatant.   |
| 3 | Repeat the treatment until the filtrate or the supernatant has exactly the same pH and conductivity as the buffer. This must be checked after each buffer change. |

**Note:**

*This method may require many changes and be time consuming when buffers of low concentrations are used.*

- |   |  |
|---|--|
| 4 | Fines removal and preparation of column packing slurry is carried out according to instruction Step 4 to 6 in the <i>Preferred method</i> above. |
|---|--|

## Large scale preparation

### Equilibration and removal of fines

Step	Action
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- |   |  |
|---|--|
| 1 | Disperse 10 kg of preswollen ion exchanger in 50 L of buffer (i.e., 1:5 ratio, w/v). The initial equilibration buffer should normally be at least 0.2 M. |
| 2 | Adjust pH with acid or alkali to $\pm 0.1$ pH units of the desired value required for chromatography.  |



Step	Action
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- |   |  |
|---|--|
| 3 | Allow the slurry to settle for 1 to 3 h (depending on the nature of the buffer and diameter of the settling vessel) and siphon off the supernatant and fines almost to the level of the settled ion exchanger. |
| 4 | Bring the slurry back to the initial volume by the addition of dilute buffer (normally in the range of 0.01 to 0.05 M) i.e., the buffer concentration required for the subsequent chromatography.              |
| 5 | After dispersion and settling, remove the supernatant by siphoning, as in Step 3. To ensure optimal performance it may be necessary to repeat step 3 and 4 until a clear supernatant is obtained.              |
| 6 | Disperse again in dilute buffer.   |
| 7 | After the ion exchanger has settled, remove the supernatant down to the volume of the settled ion exchanger plus 20%.<br><br>The ion exchanger is now ready for use in one of the separation techniques.       |

## 5 Laboratory scale chromatography

### Batch chromatography

Step	Action
1	Add a known quantity of the precycled, equilibrated and fines free ion exchanger to the crude sample solution.
2	Gently stir for a predetermined time depending on the rate at which the component(s) to be separated is adsorbed.
3	Separate the ion exchanger from the buffer by filtering or centrifuging.
4	Wash the ion exchanger with the buffer used for equilibration.
5	Elute the adsorbed component(s) in a stirred fluidized bed or in a centrifuge. Alternatively, pack the resin into a column and elute using column elution techniques described in <a href="#">Elution, on page 13</a> .

### Column chromatography

#### Column packing

**Note:** *Convection currents in the slurry must be avoided during the actual column packing!*

*It is essential that the operations are carried out as quickly as possible from pouring the slurry into the column to the stage of having a settled column bed of ion exchanger. Otherwise, convection currents in the slurry have sufficient time to be set in motion.*

Step	Action
1	Set up the column vertically in an area free of draughts, direct sunlight and heaters.
2	Fit an extension tube if the slurry volume is greater than the column volume.
3	Pour the stirred slurry into the column.
4	Allow the eluent from the column to run to waste.
5	When all slurry has been added, attach or insert the top column piece or the column adapter.
6	Pump or run the packing buffer through the column at a flow rate of at least (45 mL/hour)/cm <sup>2</sup> of the internal cross-sectional area of the column until the column bed height is constant.
7	Stop the flow of buffer into and out of the column.
8	Remove the extension tube, if fitted.
9	Mount an upper adapter (if not already done in step 5), while avoiding air entrapment. Adjust the adapter down to the bed surface.
10	Pack for additional 5 min and then re-adjust the adapter down to the bed surface.

## Equilibration

### Equilibration

Pass the starting buffer through the column until the conductivity and pH of the eluent are exactly the same as the starting buffer.

**Note:** *The reading of pH and conductivity must be exact!*

- *When the packed column is equilibrated, the eluent will be identical to the starting buffer.*
- *It is essential that readings of two consecutive eluents are not only the same, but are identical to the starting buffer.*
- *Incorrect equilibration is the most frequent cause of non-reproducible results.*

This method is suitable for column separations starting with a low concentration of buffer.

## Sample preparation and loading

- Dissolve the sample in starting buffer and adjust the pH of the solution.
- If the sample contains visible suspended matter, clarification by filtration or centrifugation is highly recommended.
- Cell extracts and ammonium sulphate precipitates should be transferred into chromatographic starting buffer by buffer exchange.
- The sample should be loaded onto the column at a controlled flow rate.

## Elution

Start the elution immediately or at a set time after sample loading. Generally, there are three methods by which chromatographic separations are achieved. These are flow-through, linear and step-wise gradient elutions.

Flow-through elution:

The buffer used in the sample and for the equilibration of the ion exchanger may also be used for elution. This may be accomplished in two ways:

If...	Then...
Target molecule is non-adsorbed	The amount of ion exchanger required depends on its capacity for the contaminants in the sample (which should be adsorbed). Column bed height is of low importance in this mode of operation.
Target molecule is adsorbed	When the mixture consists of chromatographically similar components, a relatively long column is required in order to obtain the optimum resolution. It is advisable to use only a small part (ca 5%) of the total capacity of the column. Target molecule interacts with the resin/is weakly adsorbed.

Gradient elution:

- Before elution non-adsorbed material is washed out using starting buffer.
- The composition of the elution buffer may be increased to higher ionic concentrations or adjusted to the appropriate pH, or both.
- Since the elution buffer itself is the main factor in the achievement of the separation, the amount of ion exchanger required depends on the capacity of the ion exchanger for the target compounds.

- In order to obtain higher resolution, the column length should be increased and/or the slope of the gradient should be decreased.

## 6 Large scale chromatography

### Batch chromatography

A single adsorption/desorption method can often provide a quick and easy route to a semi-purified protein fraction. The choice of anion or cation exchange resin is determined by the isoelectric point of the target molecule of interest with respect to the pH and overall ionic concentration of the system.

#### 1. Sample and resin preparation

Step	Action
1	Dissolve the sample in the starting buffer and adjust the pH and conductivity of the solution.
2	If the sample contains visible suspended matter, clarification by filtration or centrifugation is recommended.
3	Cell extracts and ammonium sulphate precipitates should be transferred into chromatographic starting buffer by buffer exchange.
4	The selected resin should be equilibrated with a buffer of the same pH and conductivity as the pretreated sample.

## 2. Sample loading

**Loading:** Disperse the equilibrated ion exchanger into the crude sample. Ionic concentration may be conveniently monitored by conductivity measurements.

**Resin excess:** In practice some excess of resin may be necessary to ensure adsorption of the target molecule. An excess amount of resin will also enhance the rate of target molecule adsorption.

**Adsorption time:** The slurry should be stirred in a cylindrical tank using a paddle-type stirrer at speeds just adequate to maintain homogeneous dispersion of the resin. Small samples of the solution may be periodically withdrawn for analysis via a filter attached to a syringe. The adsorption time required to bind 90% to 98% of the target molecule will range from about 30 to 90 min depending upon the various parameters of the particular system.

## 3. Resin recovery and washing

After adsorption is complete, recover the ion exchanger and wash with starting buffer of low ionic strength to remove non-adsorbed material. This may be achieved in two ways, the choice depending upon the scale of the process:

**Small scale processes** For relatively small scale processes requiring up to 1 kg of preswollen resin the most convenient method is to use a Buchner type filter funnel and flask of the appropriate size and filter off the resin under vacuum. The resin may be washed *in situ* on the filter with buffer (e.g., starting buffer). Only the minimum amount of buffer (1 to 2 bed volumes) should be used for this purpose, to avoid possible premature loss of the required target molecule.

**Production scale processes** For production scale processes using more than 1 kg of preswollen ion exchanger, recovery and washing of the ion exchange resin is best achieved using a basket centrifuge of either the horizontal or vertical type. The centrifuge bowl must be fitted with a suitable retaining mesh.

Both the bowl and the mesh must be constructed of chemically resistant material such as 316 stainless steel or, alternatively, must be coated with a suitable chemically inert polymer.

**Centrifugation force** After target molecule adsorption is complete, pump the slurry into the centrifuge bowl rotating at an initial force equivalent to between 250 and 500 g. A uniform continuous layer of resin with a wall thickness equivalent to a maximum of 10% of the basket diameter should be achieved. Typically, this is equivalent to an optimum loading of about 20 kg of preswollen ion exchange cellulose for a centrifuge fitted with a 53 cm (21 inch) diameter bowl.

**Washing the resin** The wall of resin should be consolidated by progressively increasing the centrifugation force up to a maximum of 5000 g. The wall of resin may then be washed with the appropriate buffer, running the centrifuge at low or moderate speeds. Minimum buffer volumes should be employed for this purpose.

## 4. Target molecule elution

**Dispersion:** After washing, re-disperse the resin in a minimum stirrable volume of the starting buffer (i.e., 1 kg of resin in 2 L of buffer).



**Increasing ionic concentration:** The ionic concentration may be progressively increased by the addition of sodium chloride until the target molecule is eluted. In many cases, a step-wise procedure may be applied whereby the salt concentration is initially increased to an intermediate level to remove weakly adsorbed molecules. Filter or spin off the resin and re-disperse in buffer adjusted to the required final salt concentration to elute the component of interest. Filter the resin in a Buchner funnel or centrifuge, depending on the process scale, and collect the eluent. In order to optimize the yield, this process must be repeated at least once and the eluates should be pooled.

**Change pH:** Alternatively, elution may be achieved by changing the pH. The pH must be changed to a value where the charges on the ion exchanger and the protein of interest are of the same sign.

**Column elution:** Target molecule elution may also be accomplished according to [3. Resin recovery and washing, on page 15](#) followed by packing into a column. Elution can then be performed in a smaller volume with higher yields compared centrifugation.

## Column chromatography

### Scaling up

To scale up from the laboratory scale to process scale, increase the cross-sectional area proportional to the increase in loading, while maintaining constant bed height and linear flow rate. A thousand-fold scale-up may therefore be achieved simply by moving from a laboratory column (1.6 cm i.d. x 16 cm) to a process column (45 cm i.d. x 16 cm) using a typical flow rate of ~35 cm/h for DE52.

## Quantity of resin required

The following table indicates quantities required per liter of packed volume and protein capacities of the various Whatman resins

Physical form and grade	Kg of resins per Liter of column volume	Kg to pack a 25 Liter column (45 cm i.d. × 16 cm)	Protein mg/dg <sup>1</sup>	Capacity g/L Bed volume
<b>Preswollen resins</b>				
<b>DE52</b>	0.9	22.5	700 <sup>2</sup>	130
<b>CM52</b>	1.1	26.5	1180 <sup>3</sup>	210
<b>Dry resins (Dry resins must be precycled before use)</b>				
<b>DE32</b>	0.24	6.0	700 <sup>2</sup>	140
<b>CM32</b>	0.21	5.5	1180 <sup>3</sup>	200

<sup>1</sup> mg/dg=milligrams of protein/dry gram of ion exchanger

<sup>2</sup> Protein capacity quoted: 0.01 M phosphate buffer, pH 8.5- Bovine Serum Albumin

<sup>3</sup> Protein capacity quoted: 0.01 M acetate buffer, pH 5.0- Lysozyme

## 1. Column packing procedure

Column packing protocols vary for different column suppliers and reference should be made to each specific column operating manuals. The guidelines for handling Whatman ion exchangers are as follows and should be used in conjunction with the column operating manuals.

Step	Action
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- |   |   |
|---|---|
| 1 | Prepare the resin according to the procedure in <i>Equilibration and removal of fines, on page 8.</i> |
|---|---|

Step	Action
2	Pack the column with a slurry of the ion exchanger. The recommended slurry consistency is 30% (w/v) based on pre-equilibrated wet weight.
3	The bed should be consolidated at constant pressure, at least 25% greater than the maximum operating pressure and typically 10 psi (0.66 bar) for a process column.

## 2. Resins equilibration

In most cases the general procedure might be employed.

However, for large scale processes, where economic factors are very important, large columns are generally run using low concentration of buffer for the initial equilibration of the resins, followed by salt gradients (e.g., sodium chloride) to achieve elution. With this type of system it may be advantageous to start the equilibration of the preswollen resin by direct dispersion in the starting buffer, followed by any necessary preliminary adjustment of pH.

**Pack column and start equilibration:** After fines removal, described in [Equilibration and removal of fines, on page 8](#) the column is packed and the equilibration of the resin is completed by passing further starting buffer through the column.

**Complete equilibration:** Equilibration is complete when the pH and conductivity of the column eluent and the starting buffer are identical.

**Buffer volume:** The volume of buffer required to achieve equilibrium will depend upon the nature of the buffer and the particular ion exchange resin employed. Equilibration volumes required for all resins are less when using buffers in which the buffering ion carries charges of the same sign as the ion exchanger (i.e., a co-ion). For example, Tris-HCl buffer for DE resins compared to sodium phosphate buffer where the buffering phosphate ion is the negative counter ion. The volume of buffer required to achieve equilibrium will vary between 2 and 5 bed volumes, depending upon the various factors identified above.

### 3. Sample loading

- Ideally the sample should be at the same pH and ionic concentration as the starting buffer. Use the same sample condition each time.
- Failure to achieve a reproducible starting point will give non-reproducible results and possibly poor separations.
- Relatively large sample volumes (5 to 500 L) are conveniently loaded through a pump and followed immediately with 2 to 5 bed volumes of starting buffer.

### 4. Gradient elution

The elution protocol developed in the laboratory should be scaled-up proportionately. A typical gradient is linear and the volume should be at least four times the bed volume of the column.

## 7 Maintenance

### Extending life time of resins

The life time of a packed column depends on the nature of the sample. The applied sample should be free of insoluble particulates and should not contain significant amounts of substances which could bind to the column resins by non-specific hydrophobic interactions.

In many cases, the column bed may be simply regenerated by increasing the salt concentration to elute all the remaining components bound by ionic interactions before re-equilibrating with the original buffer to start the next cycle.

### Cleaning-in-place

The following protocol represents a minimal exposure to sodium hydroxide (NaOH), although NaOH concentrations up to 2 M at temperatures up to 42°C may be required in applications where the resins are heavily fouled.

As an example, for a typical application with a running buffer of 0.025 M Tris-HCl, pH 7.5. The following protocol is recommended:

Step	Action
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- |   |   |
|---|---|
| 1 | Wash the column bed with 0.5 M NaOH (2 bed volumes) and let stand for 12 h at room temperature. |
| 2 | Wash with 2 bed volumes of de-ionized water.  |
| 3 | Wash with 2 bed volumes of 0.1 M Tris-HCl buffer, pH 7.5.                                       |

Step	Action
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- |   |  |
|---|--|
| 4 | Wash with 6 bed volumes of 0.025 M Tris-HCl, pH 7.5, or sufficient for equilibration of the bed. |
|---|--|

Further details are given in the Regulatory Support File.

## Sterilization and depyrogenation-in-place

All Whatman cellulose based ion exchange resins may be autoclaved for sterilization purposes. This is best carried out on a slurry of the ion exchanger buffered with a non-volatile buffer of pH 6.5 to 7.5.

The recommended conditions are: at 10 psi (70 kPa) for 15 min, or at 15 psi (100 kPa) for 10 min.

Alternatively, all Whatman cellulose products may be chemically sterilized by dispersion in 0.5 M NaOH followed by washing with sterile water. At the conclusion of the clean-in-place protocol above, the resin and column will be fully sterile provided that the final buffer is also sterile and pyrogen-free. All Whatman cellulose products may also be treated with ethanol-water mixtures containing up to 20% to 25% by volume of ethanol.

Further details are given in the Regulatory Support File.

## Degassing (Anionic exchangers)

For the most delicate work, removal of carbon dioxide absorbed by DE cellulose ion exchangers may be required to obtain reproducible results. Generally this is not necessary if the *Preferred Method* for slurry preparations is followed ([Preferred method, on page 6](#)).

Step	Action
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- |   |  |
|---|--|
| 1 | Place the ion exchange resin in the acidic component of the buffer.  |
| 2 | Check that the pH is below 4.5. If not, then a higher concentration of the acid component must be used.  |
| 3 | Apply a vacuum down to below 13 kPa (100 mm Hg pressure) with stirring, until no more bubbles are noticed, but stop before boiling occurs. This may conveniently be carried out by stirring the slurry with a magnetic stirrer in a stoppered Buchner flask connected to an aspirator. |
| 4 | Add the basic component of the buffer to give the desired pH. For more critical work, buffer solutions must be made up from CO <sub>2</sub> free water and kept free of CO <sub>2</sub> .  |

## Storage

When it is required to store ion exchange celluloses as bulk slurries or as packed columns for longer than one week, the following solutions are recommended over the pH range 3 to 11:

- For anion exchangers (DE), use 0.2% w/v benzalkonium chloride.
- For cation exchangers (CM), use 0.1% w/v 2,2' dithiobis (pyridine-N-oxide). Adjust the pH of the solution with sodium hydroxide to about 10 to 11 to better dissolve the chemical.

The chemicals may be readily and completely removed by subsequent washing with buffer or distilled or de-ionized water prior to further use.

## 8 Ordering information

### Preswollen products:

Product	Volume (kg)	Product Code
DE52	25	4057925
DE52	10	4057910
DE52	2	4057200
DE52	0.5	4057050
CM52	10	4037910
CM52	2	4037200
CM52	0.5	4037050

### Dry products:

Product	Volume (kg)	Product Code
DE32	10	4055910
DE32	0.5	4055050
CM32	10	4035910
CM32	0.5	4035050



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