Amersham Biosciences

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# SEPHADEX G-100 100 G

Article No: 17006001

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## General Product Information

ORDERING

| Pack size | Code No. |
| --- | --- |
| 100 g | 17-0060-01 |
| 500 g | 17-0060-02 |
| 5 kg | 17-0060-03 |
| 40 kg | 17-0060-07 |

¤ Thousands of documented applications.

¤ Proven reproducibility.

¤ Excellent recoveries.

¤ Economical.

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## Application area (Details)

* Fractionation and purification of proteins and peptides.

* Determination of molecular weights.

* Determination of equilibrium constants.

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## Autoclaving (Details)

In wet form (pH 7), at 120°C for 30 min.

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## Calibration (Details)

Proteins: Use LMW Gel Filtration Calibration Kit.

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## Checking the column packing (Details)

1. Run Blue Dextran at 2 mg/ml.

2. Watch the progress of a zone of this substance through the bed:
   You should see a discrete horizontal band travelling through the column.
   If not:
   A. The problem could be caused by dirty filters.

B. More likely:
An uneven bed surface or uneven packing is responsible, in which case the column must be repacked.

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## Choice of eluent (Details)

* Eluent composition does not directly influence the resolution which can be obtained:

  A. Completely uncharged substances may be eluted with distilled water.

  B. For substances carrying charged groups an eluent containing a buffer e.g. Tris-HCl, sodium phosphate is used to control pH, and an ionic strength of at least 0.02 is recommended to safeguard against possible ionic interactions with the gel matrix. NaCl can be used for this purpose.

* For the product that is to be lyophilized:
  Use volatile buffers e.g. ammonium acetate, ammonium bicarbonate or ethylenediamine acetate.

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## Cleaning (Details)

* Wash through 2 column volumes of a non-ionic detergent solution.

* Wash with 0.2 M NaOH outside the column due to swelling.

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## Colour (Details)

White.

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## Column packing (Details)

The gel suspension should reach the temperature of column operation before packing is begun.

1. Inject eluent buffer into the outlet tubing until it passes through the bed support net.

2. Close the outlet tubing, when the dead space under the net is properly filled.

3. Pour the entire slurry into the column in a single operation. (Use reservoir if necessary.)

4. Open the outlet valve.

5. Start the pump or gravity flow to initiate packing. The column should be packed at as high pressure as possible without deforming beads. The pressure should not be increased beyond 9.6

kPa (value for 2.6 x 30 cm column in aqueous buffer at room temperature. It may vary depending on eluent viscosity. With wider columns, slightly reduced maximum operating pressures must be used.)

6. Remove the reservoir when all the gel has sedimented into the column.

7. Insert the adaptor.

8. Pack at the maximum operating pressure of the gel.

9. Adjust the flow adaptor to the surface of the gel bed when the gel is thoroughly packed into the column.

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## **Composition of the gel matrix** (Details)

Dextran, cross-linked with epichlorohydrin.
Sephadex contains a small number of carboxyl groups, which at low ionic strength cause interaction between charged solutes and the matrix.

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## **Density** (Details)

We do not measure density.

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## **Equilibration** (Details)

1. Pass 2 to 3 column volumes of the aqueous buffer to be used in the separation.

2. Readjust the flow adaptor to the surface of the bed.

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## **Exclusion limit** (Details)

Dextrans

95 kD
Buffer solution: Phosphate buffer 0.05 M
- NaCl 0.15 M - NaN3 0.01% pH 7.0.

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Globular proteins

210 kD
Buffer solution: Phosphate buffer 0.05 M
- NaCl 0.15 M - NaN3 0.01% pH 7.0.

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## **Expected shelf life** (Details)

8 years.

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## Form as supplied (Details)

Dry powder.

## Fractionation range (Details)

Dextrans

1 kD - 100 kD
Buffer solution: Phosphate buffer 0.05 M
- NaCl 0.15 M - $NaN_3$ 0.01% pH 7.0.

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Globular proteins

2 kD - 120 kD
Buffer solution: Phosphate buffer 0.05 M
- NaCl 0.15 M - $NaN_3$ 0.01% pH 7.0.

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Peptides

2 kD - 120 kD
Buffer solution: Info not available.

## Major separation mechanism (Details)

According to size.

## Max. linear flow rate (Details)

50 cm/h.
Values may vary depending on column packing and eluent viscosity.

## Max. operating pressure (Details)

9.6 kPa
Value for 2.6 x 30 cm column, in aqueous buffer at room temperature.
Values may vary depending on column packing and eluent viscosity.

## Max. volumetric flow rate (Details)

4.2 ml/min
Value for 2.6 x 30 cm column, in aqueous buffer at room temperature.
Values may vary depending on column packing and eluent viscosity.

## Optimisation (Details)

* Parameters to change are:
  A. Sample volume.
    - A smaller sample volume gives better resolution.

B. Flow rate.
   ¤ Lower flow rate gives better resolution for high molecular weight components.

   ¤ The opposite may be true for small components since they diffuse more quickly.
     - The longer the separation time is, the wider the sample zones become.

C. Column length.
   ¤ The resolution of two separated zones increases as the square root of the column length.

   ¤ The effective bed height can be increased by:
     - Recycling.
     - Coupling two columns in series.

* Buffer choice and pH are normally of minor importance.

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### pH stability; operational (Details)

2 - 10

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### Range of dry bead size (Details)

40 - 120 µm

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### Range of wet bead size (Details)

100 µm - 310 µm

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### Rec. column (Details)

Long, narrow column e.g. C  16, 26 / 40 - 100 cm
                         XK 16, 26 / 40 - 100 cm

* The length of the column is decided by the resolution required, (the resolution of two separated zones in gel filtration increases as the square root of column length) and the diameter by the sample size.

* The effective bed height can be increased by recycling or using columns coupled in series.

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### Rec. linear flow rate (Details)

2 - 5  cm/h

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## Sample volume fractionation (Details)

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1 - 5 % of bed volume.

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## Solvent regain (Details)

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Distilled water   9 - 11 ml/g.

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## Storage of unused material (Details)

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4 - 25°C, dry.

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## Storage of used material (Details)

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4 - 8°C, pH 6 - 8, do not freeze.

Bacteriostatic agent (e.g. 20 % ethanol, 0.04 % sodium azide).

Storage of column:
1. Wash with 0.04 % sodium azide.

2. Put a stop screw into a bottom tubing of the column and insert the tubing from the adaptor in a Parafilm â protected vessel (e.g. test tube) with 20 % ethanol.

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## Swelling dry gel (Details)

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Swelling time: 72 h at 20°C or 5 h at 90°C.

1. Weigh out the appropriate amount of dry gel for the required bed volume.
   (Approx. bed volume: 15 - 20 ml/g dry gel.)

2. Add enough buffer to equal the total volume of the column plus 30 % more.

3. Stir - excessive stirring should be avoided as it may break the beads.
   DO NOT USE MAGNETIC STIRRERS!!!!!
   The process is accelerated by using a boiling water bath, which also serves to deaerate the buffer.

4. Decant the supernatant (after complete swelling).

5. Add back buffer to make a suspension.
   This should be fairly thick, (about 75 % settled gel) and de-gassed.

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## Swelling of dry material (Details)

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Dimethyl sulphoxide      45 ml/g

Distilled water 15 - 20 ml/g

Saline 19 ml/g

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## **Temperature stability** (Details)

120°C.
Dry gel should not be heated to more than 120°C as it will caramelize.

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## **Toxicity data** (Details)

We do not have any toxicity data for our gels.
They are intended for in vitro use only.

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## **2-chloroethanol 30 %** (Stability)

Should be OK.

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## **2-mercaptoethanol 0.1 M** (Stability)

OK
OK 60 min, 60°C.

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## **Acetic acid 50%** (Stability)

Not recommended.

Degradation of the gel.

"It seems probable that solubilization of the more highly cross-linked gels (than Sephadex G-150) by strong formic and acetic acids also occurs but has escaped notice, because the most commonly applied procedures for examining peptide containing eluents would not have detected the presence of the carbohydrate."

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## **Acetic acid 1 M** (Stability)

OK
OK equilibration/elution.

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## **Alkaline solutions** (Stability)

OK

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**Aqueous salt solutions** (Stability)

OK

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**Brij 58 16 mg/ml** (Stability)

Should be OK.

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**CHAPS 30 mM** (Stability)

OK
OK for equilibration/elution.

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**Chloroform/methanol (3:1)** (Stability)

OK
OK for equilibration/elution.

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**Dextranase** (Stability)

Should be avoided.

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**Dimethyl sulphoxide** (Stability)

OK
OK for equilibration/elution.

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**Dithiothreitol 1 mM** (Stability)

Should be OK.

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**Empigen BB 1 %** (Stability)

Should be OK.

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**Formamide 14 M** (Stability)

Should be OK.

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### **Formic acid 10%** (Stability)

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OK
OK equilibration/elution.

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### **Formic acid 50 %** (Stability)

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Use with care.

Conflicting reports.

1. OK for equilibration/elution.

2. Degradation of the gel.

"It seems probable that solubilization of the more highly cross-linked gels (than Sephadex G-150) by strong formic and acetic acids also occurs but has escaped notice, because the most commonly applied procedures for examining peptide, containing eluents would not have detected the presence of the carbo- hydrate."

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### **Glucose 0.1 M** (Stability)

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OK
OK elution.

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### **Glycine-HCl 0.1 M pH 1.0** (Stability)

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OK
OK elution.

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### **Guanidine hydrochloride 6 M** (Stability)

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OK
OK equilibration/elution.

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### **Hydrazine 80 %** (Stability)

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Should be OK.

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### **Hydrochloric acid 0.02 M** (Stability)

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OK

The gel was well soaked in the cold room in 0.02 M HCl.

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**Methanol 50%** (Stability)

OK
OK elution.

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**Organic solvents** (Stability)

OK

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**Oxidizing agents** (Stability)

Should be avoided.

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**Piperidine 1 M** (Stability)

Should be OK.

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**Propionic acid 1 M** (Stability)

OK
OK equilibration/elution.

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**Sodium cholate 15 mM** (Stability)

OK
OK for equilibration/elution.

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**Sodium deoxycholate 16 mg/ml** (Stability)

Should be OK.

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**Sodium dodecyl sulphate 3 %** (Stability)

Should be OK.

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**Strong acids** (Stability)

Should be avoided.

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### **Triton X-100 30 mM** (Stability)

OK
OK for equilibration/elution.

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### **Urea 8 M** (Stability)

OK
OK equilibration/elution.

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### **Weakly acidic solutions** (Stability)

OK

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