# Q Sepharose® Fast Flow

***INSTRUCTIONS***

## Important user information

Please read these instructions carefully before using Q Sepharose Fast Flow media. Should you have any comments on this instruction manual, we will be pleased to receive them at:

Amersham Biosciences AB

S-751 82 Uppsala

Sweden

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71-5003-05

Edition AA

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# 1. Introduction

Q Sepharose Fast Flow is one of Amersham Biosciences' range of **BioProcess Media**, separation media that meet the demands of today's industrial bioprocessing for reproducibility, scalability, chemical and physical stability, security of supply and prompt delivery.

These instructions contain information on media characteristics, column packing and evaluation, maintenance, and trouble-shooting.

To ensure best peformance and trouble-free operation, please read these instructions before using Q Sepharose Fast Flow.

# 2. Media characteristics

Q Sepharose Fast Flow is a strong anion exchanger. The ion exchange group is a quartenary amine group, see Fig. 1.


***Fig. 1.*** The ion exchange group of Q Sepharose Fast Flow.

The highly cross-linked agarose base matrix gives the media chemical and physical stabilities. Characteristics such as capacity, elution behaviour and pressure/flow rate are unaffected by the solutions commonly used in process chromatography and cleaning procedures, see Table 1.

High physical stability gives exceptional flow characteristics and low back pressures. Linear flow rates of 400–700 cm/h through a bed height of 15 cm at a pressure of 1 bar are typical, see Figure 2. The high rigidity of the matrix minimizes volume variations during change of pH or ionic strength.

The titration curve in (fig 3) shows the broad pH working range of Q Sepharose Fast Flow, i.e. the pH range in which the Q group is charged.

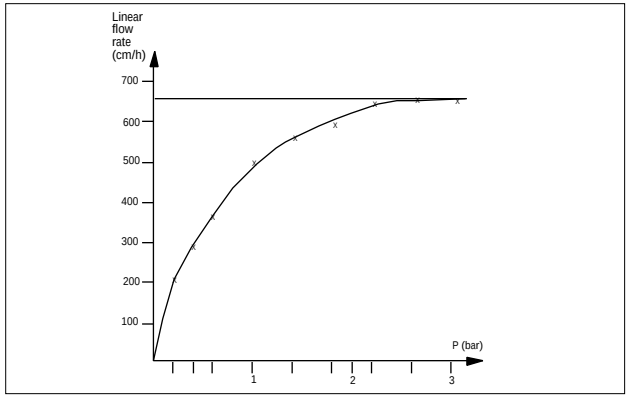


***Fig. 2.*** A typical pressure/flow rate curve for Sepharose Fast Flow ion exchangers.

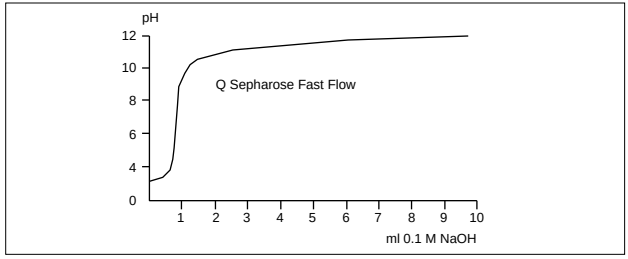


***Fig 3.*** Q Sepharose Fast Flow is charged over wide pH range.



***Table 1.*** Media characteristics.

| **Property** | **Description** |
|---|---|
| Ion exchange type | Strong anion |
| Total ionic capacity | 0.18–0.25 mmol/ml media |
| Exclusion limit | $4x10^6$ (globular proteins) |
| Matrix | Cross-linked agarose, 6% |
| Bead form | Spherical, 45–165 µm |
| Flow rate | 400–700 cm/h* |
| Working temperature | 4–40 °C |
| Working pH | see Fig. 3. |
| pH stability | 1–4 (short-term, CIP)<br>2–12 (long-term) |
| Chemical stability | All commonly used |
| | aqueous buffers |
| | 1 M NaOH |
| | 8 M urea |
| | 6 M guanidine hydrochloride |
| | 70% ethanol |
| *The following should be avoided* | |
| | Oxidizing agents |
| | Long exposures |
| | (1 week, 20 °C) |
| | to pH <4 |

*15 cm bed height, 1 bar, 25 °C, XK 50/30 column

## 3. Column packing guidelines

Q Sepharose Fast Flow is supplied in suspension in 20% ethanol. Decant the 20% ethanol solution and replace with starting buffer before use.

### 3.1 Recommended columns

BPG, variable bed, glass columns: inner diameters from 100–450 mm, bed volumes from 2.4–43 litres; bed height max 30 cm (27 cm for BPG 450).

BioProcess Stainless Steel fixed bed columns: inner diameters from 400–1400 mm; fixed bed volumes from 19–230 litres; fixed bed height 15 cm.

Process Stack column 370: inner diameter 370 mm, fixed bed volume of 16 litres at fixed bed height of 15 cm.

INdEX variable bed columns: inner diameters from 70–200 mm; bed volumes up to 19.4 litres; bed heights max 60 cm.

### 3.2 Packing recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully.

Sepharose Fast Flow ion exchangers are easy to pack since their rigidity allows the use of high flow rates, see Figure 2. Four types of packing methods suitable for Q Sepharose Fast Flow are listed below.

- Pressure packing (for columns with adaptors).
- Combined pressure/suction packing (for medium sized columns with fixed bed heights).
- Suction packing (for large columns with fixed bed heights).
- Hydraulic pressure packing.



How well the column is packed will have a major effect on the result of the separation. It is therefore very important to pack and test the column according to the following recommendations.

Begin the packing procedure by determining the optimal packing flow rate. Guidelines are given for determining the optimal packing flow rates for columns with adaptors and fixed bed heights.

### 3.3 Determining optimal packing flow rates

The optimal packing flow rate is dependent on temperature, column size and type, media batch and volume. Consequently, the optimal packing flow rate must be determined empirically for each individual system.

To determine the optimal packing flow rate, proceed as follows:

1. Calculate the exact amount of media needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of media required per litre packed volume is approximately 1.15 litres sedimented media.
2. Set up the column as for packing according to the instructions in section 3.2.
3. Begin packing the media at a low flow rate (30 cm/h).
4. Increase the pressure in increments and record the flow rate when the pressure has stabilized. Do not exceed the maximum pressure of the column, or the maximum flow rate for the media.
5. The maximum flow rate is reached when the pressure/flow curve levels off or the maximum pressure of the column is reached. Stop the packing and do not exceed this flow rate. The optimal packing flow rate/pressure is 70–100% of the maximum flow rate/pressure.
6. Plot the pressure/flow rate curve as in Fig. 2 and determine the optimal packing flow rate.

The operational flow rate/pressure should be <70% of the packing flow rate/pressure.

**Note:** For BPSS columns, pack the column according to instructions in section 3.6.

### 3.4 Pressure packing

**BPG columns**

BPG columns are supplied with a movable adaptor. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at constant flow rate (or back pressure).

1. Pour some water (or packing buffer) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2. Mix the packing buffer with the medium to form a 50–70% slurry. (Sedimented bed volume/slurry volume = 0.5–0.7.)

   Pour the slurry into the column. Insert the adaptor and lower it to the surface of the slurry, making sure no air is trapped under the adaptor. Secure the adaptor in place.
3. Seal the adaptor O-ring and lower the adaptor a little into the slurry, enough to fill the adaptor inlet with packing solution.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or medium.
5. When the medium has settled, mark the bed height on the column tube, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adaptor to about 0.5–1.0 cm from the medium surface.
6. Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between medium surface and adaptor when the medium has stabilized.
7. Close the bottom valve, stop the pump, disconnect the column inlet and push the adaptor down to approximately 3 mm below the mark on the column tube, without loosening the adaptor O-ring. The packing solution will flush the adaptor inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).



### 3.5 Combined pressure/suction packing

**Process stack (PS 370) Column**

The Process Stack Column is supplied with fixed end-pieces and a fixed bed height of 15 cm. It is packed by a combined pressure/suction technique.

1. Fit an extra column section on top of the column tube, for use as a packing device.
2. Pour some water (or packing buffer) into the column. Make sure that there is no trapped air under the bottom net. Leave about 2 cm of liquid in the column.
3. Pour the slurry into the column. Add buffer to within 1–2 cm of the rim of the upper section. Stir gently to give a homoge neous slurry. Add buffer until level with the upper rim and secure the lid in place.
4. Connect a pump and a pressure meter and start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or medium.
5. When the medium has stabilized, the top of the bed should be exactly level with the top of the column tube. At this point, exclude the buffer tank from the system by simultaneously switching the valve at the column outlet and the valve on the suction side of the pump, as shown in Fig. 4. Packing buffer is now recirculated through the system . If, when stabilized, the packed bed is not exactly level with the top of the column, add or remove slurry. Always stir the slurry thoroughly before packing.

6. Keeping the pump running, disconnect the column inlet from the lid and direct it to waste. The packing solution in the packing section can be removed by suction through the bed.
7. While the packing section is being emptied, loosen the bolts holding the column and the packing section together so that the packing section can be removed. During this operation, manually press down on the packing section to prevent leakage between the two sections.
8. When the packing solution is within 5–8 mm of the bed surface, close the valve at column outlet, stop the pump, quickly remove the packing section and replace it with the lid. Manually press down on the lid while it is secured in place.

This final operation should be completed as quickly as possible because the bed will expand when the flow stops.

9. Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

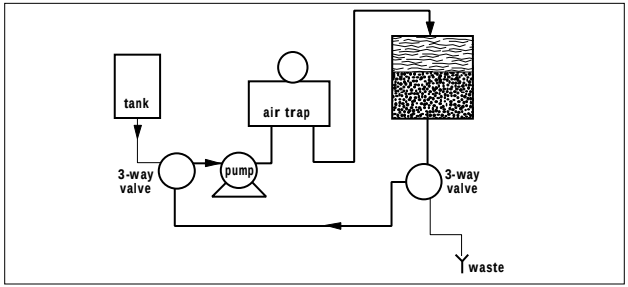


***Fig. 4.*** Equipment set up for pressure/suction packing.



## 3.6 Suction packing

**BioProcess Stainless Steel (BPSS) Columns**

BioProcess Stainless Steel Columns are supplied with fixed end pieces. They are packed by suction, i.e. by sucking packing solution through the chromatographic bed at a constant flow rate.

1. Fit a packing device on top of the column tube.
2. Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 23 cm of liquid in the column.
3. Mix the packing buffer with the medium to form a 50% slurry (sedimented bed volume/slurry = 0.5). Pour the slurry into the column. Stir gently to give a homogeneous slurry.
4. Connect the column outlet valve to the suction side of a pump and start packing the bed by suction through the bed at the predetermined flow rate, see Fig. 4. Keep the flow rate constant during packing.
5. When the bed has stabilized, the top of the bed should be just below the junction of the column and the packing device.

If, when stabilized, the level of the bed is incorrect, add or remove slurry. Always stir the slurry thoroughly before packing.

6. Just before the last of the solution has entered the packed bed (before the surface starts to dry), close the valve at the column outlet, stop the pump, quickly remove the packing device and replace it with the lid.

This final operation should be completed as quickly as possible because the bed will expand when the flow stops.

7. Start pumping buffer with upward flow through the column to remove any air bubbles trapped under the lid.

## 3.7 Hydraulic packing

**INdEX Columns**

INdEX columns are supplied with a hydraulic function which allows an extremely simple, rapid and reproducible packing

procedure to be used. The medium is packed at the same time as the adaptor is lowered into position at the correct pressure.

The adaptor is pushed down by a constant hydraulic pressure, forcing water through the slurry and compressing it so that a packed bed is gradually built up. The hydraulic pressure can be generated using a pump and a pressure relief valve.

1. Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2. Pour the slurry carefully into the column. Fill the column with buffer solution up to the edge of the glass tube. Mix the slurry and buffer solution. Allow the medium to settle to below the bevel (G) on the glass tube, see Fig. 3.
3. Rest the adaptor against the bevel (G) on the glass tube. Tilt the lid slightly to avoid trapping air bubbles under the net when mounting it on the column. Lower and secure it in place.
4. Connect a pump to the inlet of the hydraulic chamber (A) in-line with a manometer and a pressure relief valve, between the pump and the hydraulic chamber. The manometer should be placed after the valve in the direction of the flow.
6. Open the hydraulic inlet (A) and the hydraulic outlet (C). Start the pump and flush the hydraulic chamber (E) free of air and any residual medium.
7. Close (C) and open the elution inlet/outlet (B) to expel trapped air in the adaptor net.
8. Close (B) and open the elution inlet/outlet (D) to start packing. Apply a pre-defined constant hydraulic packing pressure.
9. When the bed has finally settled (no flow at the column outlet), stop the packing procedure by closing (A) and (D). The adaptor stops moving when the hydraulic force, acting downwards is equal to the mechanical force of the bed, expressed upwards.



10. To unpack the column, connect the outlet from the pump to (B) and open (C) while keeping (D) closed. This will cause the adaptor to rise from the bed surface.

**Note:** The hydraulic pressure used for packing is not comparable to the back-pressure generated when packing with a pump or pressure vessel. When using hydraulic pressure packing, the bed is mechanically compressed during the last part of the procedure. As a result, the flow properties of the packed bed will be limited by this mechanical compression.

At any flow rate, the pressure drop over the bed under running conditions is higher than expected from the hydraulic pressure applied during packing.

It is therefore important to carefully optimize the hydraulic packing pressure in order to achieve the same flow properties as for columns packed with conventional techniques using a pump.

When packing Sepharose 4 Fast Flow media in INdEX columns to bed heights of 15 and 30 cm, the optimal hydraulic packing pressure is between 0.7 bar and 0.8 bar. The final mechanical compression at the end of the packing procedure should be about 5 mm. The degree of mechanical compression is critical for the flow properties of the packed bed.

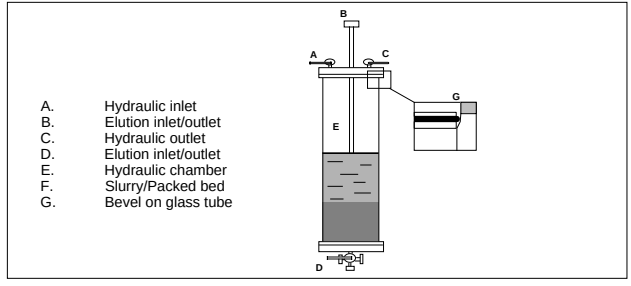


***Fig. 5.*** Schematic representation of INdEX column with a 4-port (2-way) valve mounted at the bottom outlet.

## 4. Evaluation of packing

To check the quality of the packing and to monitor this during the working life of the column, column efficiency should be tested directly after packing, at regular intervals afterwards, and when separation performance is seen to deteriorate.

The best method of expressing the efficiency of a packed column is in terms of height equivalent to a theoretical plate, HETP, and the peak asymmetry factor, $A_s$. These values are easily determined by applying a sample such as 1% acetone solution to the column. (Coloured compounds and salt solutions should be avoided since they may interact with the media.)

It is of utmost importance to realize that the calculated plate number will vary depending on the test conditions and it should therefore be used as a reference value only. It is also important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc. will influence the results.

For optimal results, the sample volume should be at maximum 2.5% of the column volume and the linear flow rate between 15 and 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as part of the acceptance criteria for column use.

*Method for measuring HETP and $A_s$*

To avoid dilution of the sample, apply it as close to the column inlet as possible.

| **Conditions** | |
|---|---|
| Sample volume: | 1.0% of bed volume |
| Sample conc.: | 1.0% (v/v) acetone in water, 2.0 M NaCl or10x buffer |
| Eluent: | water, 0.5 M NaCl in water or dilute buffer |
| Flow rate: | 30 cm/h |
| Detection: | |
| Acetone: | UV 280 nm; |
| NaCl, buffer: | Conductivity |



Calculate HETP and $A_s$ from the UV curve (or conductivity curve) as follows:

$$HETP = L/N$$

and

$$N = 5.54(V_e/Wh)^2$$

where $V_e$ = Peak elution distance

$W_h$ = Peak width at half peak height

L = Bed height (cm)

N = Number of theoretical plates

$V_e$ and $W_h$ are in the same units.

To facilitate comparison of column performance the concept of reduced plate height is often used.

The reduced plate height is calculated as

$$\frac{HETP}{d}$$

where d is the diameter of the bead. As a guideline, a value of <3 is normally acceptable.

The peak should be symmetrical, and the asymmetry factor as close as possible to 1 (values between 0.8–1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation

$$A_s = b/a$$

where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

Figure 6 shows a UV trace for acetone in a typical test chromatogram in which the HETP and $A_s$ values are calculated.

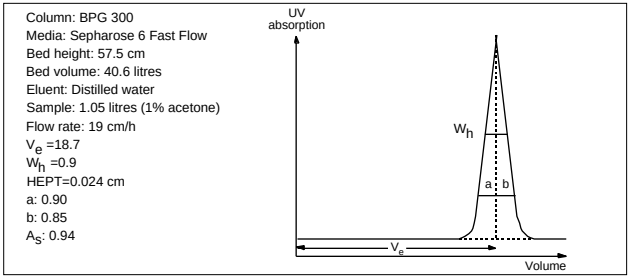


***Fig. 6.*** UV trace for acetone in a typical test chromatogram showing the HETP and $A_S$ value calculations.



## 5. Maintenance

For best performance from Q Sepharose Fast Flow over a long working life, follow the procedures described below.

*Equilibration*

After packing, and before a chromatographic run, equilibrate with working buffer by washing with at least 5 bed volumes.

*Regeneration*

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g. 1 M NaCl in buffer) or by increasing pH. Regenerate the media by washing with at least 5 bed volumes of buffer, or until the column effluent shows stable conductivity and pH values.

*Cleaning-In-Place*

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Such contamination is especially likely when working with crude materials. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of SP Sepharose Fast Flow.

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 1–5 separation cycles.

*CIP protocol*

| | |
|---|---|
| Ionically bound proteins | Wash with 0.5 column volumes of filtered 2 M NaCl. Contact time 10–15 min. Reversed flow direction. |
| Precipitated, hydrophobically bound proteins or lipoproteins | Wash with 1 M NaOH at 40 cm/h. Contact time 1–2 hours. |
| Lipids and very hydrophobic proteins | Wash with 2–4 column volumes of 0.5% non-ionic detergent (e.g. 1 M acetic acid). Contact time 1–2 hours. Reversed flow direction. Alternatively wash with 2–4 column volumes of up to 70% ethanol* or 30% isopropanol. Contact time 1–2 hours. Reversed flow direction. |

(**Note:** Specific regulations may apply when using 70% ethanol since it can require the use of explosion-proof areas and equipment.)

*Sanitization*

To reduce microbial contamination in the packed column, sanitization using 0.5–1.0 M NaOH with a contact time of 1 hour is recommended.

The given CIP protocol will, in addition to removing bound contaminants, also sanitize the media effectively.

*Sterilization*

Autoclaving is the only recommended sterilization treatment. Equilibrate the media with 0.5 M NaCl, pH 7. Dismantle the column and autoclave the media at 120 °C for 30 minutes.

Sterilize the column parts according to the instructions in the column manual. Re-assemble the column, then pack and test it as recommended.

*Storage*

Unused media can be stored in the container at +4 °C. Ensure that the screw-top is fully tightened. Packed columns should be equilibrated in working buffer containing 20% ethanol to prevent microbial growth.



## 6. Process optimization

For details, please consult the handbook ”Ion exchange Chromatography: Principles and Methods” available from Amersham Biosciences, code number 18-1114-21, section II.

## 7. Trouble-shooting guide

*High back pressure*

1.Check that all valves between the pump and the collection vessel are fully open.

2.Check that all valves are clean and free from blockage.

3.Check if equipment in use up to and after the column is generating any back pressure. (For example valves and flow cells of incorrect dimensions.)

4.Perform CIP to remove tightly bound material from the media.

5.Check column parts such as filters, nets etc., according to the column instruction manual.

*Unexpected chromatographic results*

1.Check the recorder speed/signal.

2. Check the flow rate.

3.Check the buffers.

4.Check that there are no gaps between the adaptor and the media bed, or back mixing of the sample before application.

5.Check the efficiency of the column packing, see page 14.

6.Check if there have been any changes in the pretreatment of the sample.

*Infections*

1.Check the connections and prefilters.

2.Check the in-going components such as buffers, sample components, etc.

3.Check that the column has been properly sanitized.



*Trapped air*

1.Check that the buffers are equilibrated to the same temperature as the packed column.

2.Check that there are no loose connections or leaking valves.

If air has entered the column, the column should be repacked. However, if only a small amount of air has been trapped on top of the bed, or between the adaptor net and head, it can be removed by pumping eluent in the opposite direction. After this, check the efficiency of the packed bed (see page 14) and compare the result with the original efficiency values.

***Table 2.*** How the experimental conditions of an ion exchange cycle affect the main parameters of a separation step. Optimizing the key parameter helps meet the goal of the step and contributes to an efficient and economic separation scheme.

| **Conditions** | **Main goal of a particular step** | | | |
|---|---|---|---|---|
| | **Selectivity** | **Efficiency (Theoretical Plates)** | **Capacity** | |
| | | | Throughput | Binding capacity |
| Adsorption pH | Great influence | | Influence | Great influence |
| Desorption pH | Great influence | | | |
| Adsorption conductivity | | | | Large increase |
| Gradient shape | Influence | Influence | Influence | |
| Increase flow rate | | Decrease | Large increase | Decrease |
| Increase bed height | Increase | Increase | Decrease | |
| Increasing column diameter | | | Large increase | |
| Decreasing particle diameter | | Large increase | | Increase |

## 8. Ordering information

| Product | Pack size | Code No. |
|---|---|---|
| Q Sepharose Fast Flow | 25 ml | 17-0510-10 |
| | 300 ml | 17-0510-01 |
| | 5 litres | 17-0510-04 |
| | 60 litres | 17-0510-60 |

All bulk media products are supplied in suspension in 20% ethanol.

| Handbooks | |
|---|---|
| Ion Exchange Chromatography: | |
| Principles and Methods | 18-1114-21 |
| Process Chromatography: | |
| A practical Guide | 18-1060-48 |

### Columns

For information about process scale columns, please ask for the following Data Files.

| Data File | Code No |
|---|---|
| BPG 100, 140, 200, 300 | 18-1115-23 |
| BPG 450 | 18-1060-59 |
| INdEX | 18-1115-61 |
| BioProcess Stainless Steel | 18-1121-08 |
| Process Stack (PS 370) | 18-1020-41 |

For additional information, including Data File, application references and Regulatory Support File, please contact your local Amersham Biosciences representative.

Printed in Sweden by Snits & design AB / TryckJouren Feb., 1997