SOURCE

# SOURCE 15PHE PE 4.6/100

## Quick Information

SOURCE 15PHE PE 4.6/100 is a pre-packed PEEK column for rapid preparative hydrophobic interaction chromatography of proteins and oligonucleotides.

**Read the Instruction**

The instructions on this page will help you get started quickly with your new column.
The other side gives more in-depth information on optimisation and trouble-shooting.

**Column data**

| | | |
|---|---|---|
| Matrix: | Polystyrene/divinyl benzene | |
| Ligand: | Phenyl | |
| Bead form: | Rigid, spherical porous, monodisperse | |
| Particle size: | 15 µm | |
| Capacity: | At least 40 mg albumin/column | |
| pH stability: | **Long term** | **Short term** |
| | 2–12 | 1–14 |
| Temperature: | **Regular use** | **Storage** |
| | +4 to +40 °C | +4 to +30 °C |
| Pressure over the column | **Regular use** | **Do not exceed** |
| | 0.25–2.5 MPa | 4 MPa |
| Flow rate: | **Regular use** | **Maximum** |
| (water at room temperature) | 0.5–2.5 ml/min | 5.0 ml/min |

**First time use**

Equilibration of the column before initial use or after long term storage or changing buffers:

1. 8 ml elution buffer.
2. 8 ml starting buffer.

| | |
|---|---|
| Starting buffer: | 50 mM phosphate buffer, 1.5 M (NH4)2SO4, pH 7.0 |
| Elution buffer: | 50 mM phosphate buffer, pH 7.0 |
| Flow rate: | 1.0 ml/min |

**Note:** Before connecting the column, start the pump and remove all air in the system, in particular in tubings and valves.

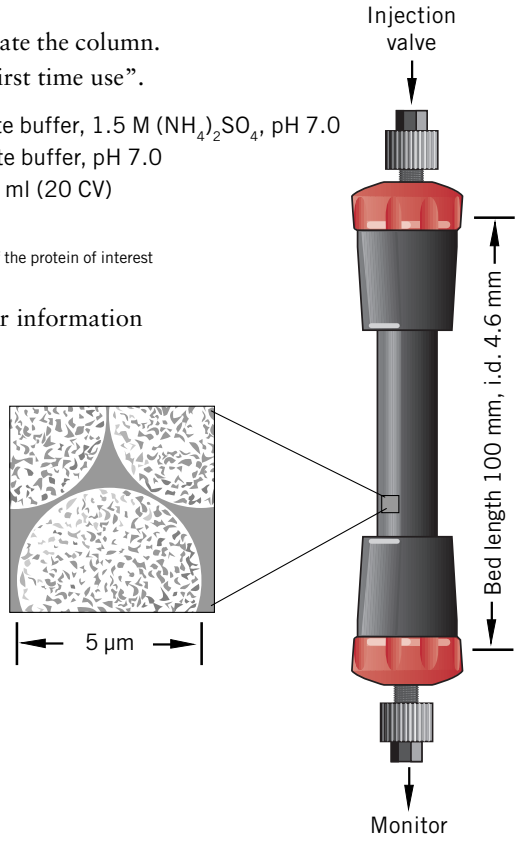
**Try these conditions first**

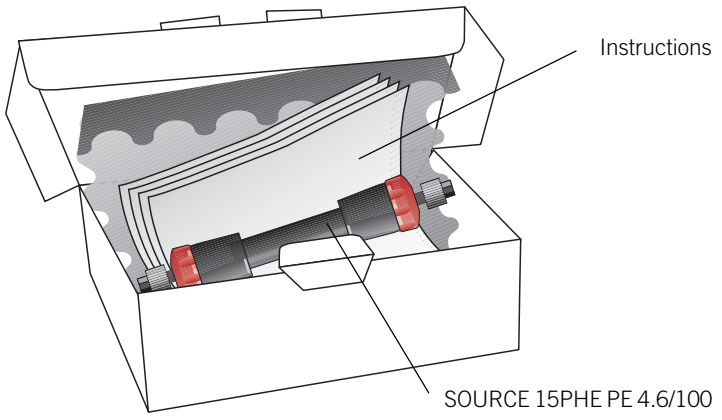
Before applying the sample, equilibrate the column.
Proceed according to the section "First time use".

| | |
|---|---|
| Starting buffer*: | 50 mM phosphate buffer, 1.5 M $(NH_4)_2SO_4$, pH 7.0 |
| Elution buffer: | 50 mM phosphate buffer, pH 7.0 |
| Gradient: | 0-100% B in 33 ml (20 CV) |
| Flow rate: | 2.0 ml/min |

* Use a lower concentration of ammonium sulphate if the protein of interest begins to precipitate at this concentration.

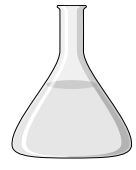
Read the back of this instruction for information on optimising a separation.



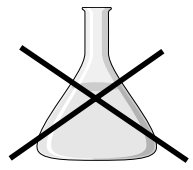

**Buffer and solvent resistance**

De-gas and filter all buffers through a 0.22 µm filter.



**Daily use**
Aqueous solutions pH 2–12
Urea, up to 8 M
Acetonitrile, up to 30% in aqueous buffers

**Cleaning**
Acetonitrile, up to 30%
Ethanol, up to 100%
Methanol, up to 100%
2-propanol, up to 100%
Hydrochloric acid, up to 1 M
Sodium hydroxide, up to 2 M
Acetic acid, up to 50%
Guanidine hydrochloride, up to 8 M
Anionic, cationic and non-ionic detergents



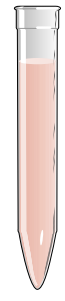
**Avoid**
Unfiltered solutions
Oxidising agents

**Sample requirements/recommendations**

| | |
|---|---|
| Recommended sample load: | ≤ 40 mg protein/column |
| Sample preparation: | Filtered through a 0.22 µm filter or centrifuged at 10 000 g for 10 min |
| Temperature*: | Ambient |

The sample should be dissolved in starting buffer.

*Hydrophobic interactions increases with increased temperature. Results achieved at room temperature may therefore not be reproduced in cold room, or vice versa.



## In Depth Information

**Delivery/storage**

The gel is delivered in 20% ethanol. If the column is to be stored for more than two days after use, wash the column with at least 8 ml distilled water and then equilibrate it with at least 8 ml 20% ethanol.



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## Optimisation

**Standard protocol**

A first run can be performed as described in the section "Try these conditions first". If the result is unsatisfactory, consider the following:

| Action | Effect |
|---|---|
| Change pH/buffer* | Weaker/stronger binding |
| Change salt* | Selectivity change |
| Decrease the sample load | Improved resolution |
| Decrease the flow rate | Improved resolution |
| Use a shallower gradient | Improved resolution, but broader peaks and decreased concentration in fractions |

* Refer to the section "choice of buffer"

**Note:** Hydrophobic interaction increases with increased temperature. Results achieved at room temperature may therefore not be reproduced in cold room, or vice versa.

## Choice of buffers

In general, the adsorption process is often more selective than the desorption process and it is therefore important to optimize the starting "binding" buffer conditions with respect to:

- pH
- salt concentration
- type of salt

The combination of salt and pH can be manipulated to give optimum selectivity. Optimal conditions differ from application to application and are best established by running linear gradients and by varying the salt concentration and pH in the starting buffer.

The buffers given in the section "Try these conditions first" are recommended as the first choice.

The Hofmeister series, Table 1, gives approximate guidelines in choosing the type of salt to use. The most efficient salts are normally ammonium sulphate (up to 2 M) and sodium sulphate (up to 1 M) but also "weaker" salts such as sodium chloride (up to 4 M) can be considered.

**Table 1.** The Hofmeister series.

← Increasing binding effect

Anions: $PO_4^{3-} > SO_4^{2-} > CH_2COO^- > Cl^- > Br^- > NO^{3-} > ClO^{4-} > I^- > SCN^-$

Cations: $NH^{4+} > Rb^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^+ > Ca^{2+} > Ba^{2+}$

← Increasing binding effect

For more information, please refer to the handbook "Hydrophobic Interaction Chromatography, Principles and Methods" available from Amersham Biosciences.

## Column cleaning

**Regular cleaning:**

After each run, wash the column with 8 ml distilled water.

Before next run, re-equilibrate the column with at least 8 ml starting buffer until the UV base-line and pH/conductivity values are stable.

**More rigorous cleaning:**

Reverse the flow direction and run at a flow rate of 0.2 ml/min with the following sequence of solutions:

1. 5 ml 30% isopropanol
2. 5 ml distilled water
3. 5 ml 1 M NaOH
4. 5 ml distilled water.

**Note:** Do not store the column in 1 M NaOH.

Depending of the nature of the contaminants, the following cleaning solutions may also be appropriate:

70% ethanol
30% acetonitrile
2 M NaOH including 1 M NaCl
0.5% non-ionic detergent in 1 M acetic acid

Always rinse with 3 ml distilled water when any of the above cleaning solutions have been used. If detergents have been used, rinse with at least 8 ml of 70% ethanol followed by 5 ml distilled water before equilibrating the column.

If column performance is still not restored, inject a solution of 1 mg pepsin/ml in 0.1 M acetic acid including 0.5 M NaCl and leave overnight at room temperature or 1 hour at 37 °C. Depending on the contamination, other enzymes can also be used, e.g. DNAase. After enzymic treatment, repeat step 1–4 in the rigorous cleaning procedure described above. Wash with elution buffer before equilibration with starting buffer and sample application.

## Trouble-shooting

| Symptom | Remedy |
|---|---|
| Increased back-pressure the column | Using reverse flow at 0.5 ml/min, pump 15 of ml elution buffer through the column. Then return to normal flow direction and run for 10 minutes at 2.0 ml/min. |
| Loss of resolution and/or decreasedsample recovery | Follow the procedure described in the section "More rigorous cleaning". |
| Air in the column | Reverse the flow direction and pump 20 ml of well de-gassed starting buffer at a flow rate of 0.5 ml/min. |

DO NOT OPEN THE COLUMN!

## Column performance control

We recommend checking the column performance at regular intervals. The function of the column can be checked as described in Figure 1.

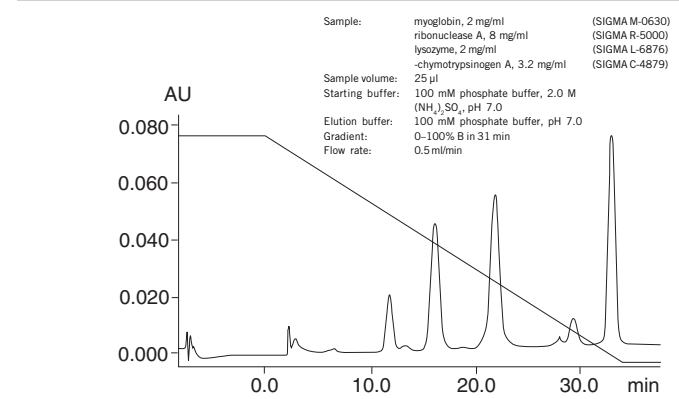


**Fig 1.** Typical chromatogram from a function test of SOURCE 15PHE PE 4.6/100.

## Accessories

| Designation | No. per pack | Code No. |
|---|---|---|
| On-line Filter | 1 | 18-1118-01 |
| Handbook: "Hydrophobic Interaction Chromatography, Principles and Methods" | 1 | 18-1020-90 |

## Ordering information

| Designation | No. per pack | Code No. |
|---|---|---|
| SOURCE 15PHE PE 4.6/100 | 1 | 17-5071-01 |



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