Procainamide **Sepharose 4 Fast Flow**

CUSTOM DESIGNED MEDIA

Procainamide Sepharose™ 4 Fast Flow is an affinity medium designed to purify butyrylcholinesterase, a plasma protein involved in the regeneration of synapses of the nervous system (1). Lack of butyrylcholinesterase increases sensitivity to succinyl choline exposure. There is also strong evidence that butyrylcholinesterase is the major detoxicating enzyme of cocaine.

Key characteristics of Procainamide Sepharose 4 Fast Flow include:

- Long spacer arm makes ligand easily available for binding target molecules
- Excellent scalability
- Ideal for industrial purification of butyrylcholinesterase

Characteristics

Procainamide Sepharose 4 Fast Flow is based on a highly cross-linked 4% agarose matrix that enables the rapid processing of large sample volumes. The small Procainamide ligand is covalently bound to the base matrix via a long hydrophilic spacer arm (Fig 1) to make it easily available for interaction with, for example, butyrylcholinesterase. Table 1 summarizes the main characteristics of Procainamide Sepharose 4 Fast Flow.

Principles of operation

Affinity chromatography exploits an immobilized ligand's ability to adsorb a specific molecule or group of molecules under certain binding conditions and then desorb them under suitable elution conditions. Both types of condition depend on the target molecule, feed composition and chromatography medium, and must be studied together with other chromatographic parameters (e.g., sample load, flow velocity, bed height, regeneration, cleaning-in-place) to establish the conditions that bind the largest amount of target molecule in the shortest time and with the highest product recovery.

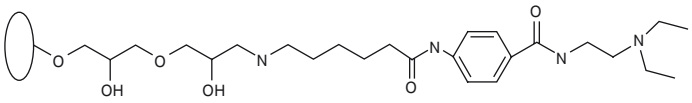

Fig 1. Partial structure of Procainamide Sepharose 4 Fast Flow. The long spacer arm facilitates target molecule binding.

Typical binding conditions for affinity chromatography with Procainamide Sepharose 4 Fast Flow are 100 mM NaCl in 20 mM phosphate buffer at pH 7.0. After washing the column with binding buffer to get rid of non-bound proteins, the target molecule can be eluted by increasing the strength of the salt gradient to approximately 1 M NaCl (2).

A column bed height of 5 to 15 cm will allow the use of high flow velocities. As a guide, Figure 2 shows pressure/flow velocity curves for the Sepharose 4 Fast Flow base matrix in a laboratory K 50/30 column and a process-scale BP 113 column.

Table 1. Main characteristics of Procainamide Sepharose 4 Fast Flow

Average particle size	90 µm
Matrix	Macroporous cross-linked 4% agarose
Ligand	Procainamide
Ligand density	20 to 26 µmol/mL medium
Flow velocity	150 to 250 cm/h, 25 cm bed height, 0.1 MPa distilled water in an XK 50 column
pH stability (long-term)	2 to 12
pH stability (short-term)	1 to 14

Like other affinity chromatography media based on the Sepharose Fast Flow matrix, purifications run on Procainamide Sepharose 4 Fast Flow are easy to scale up and operate in an industrial processing environment. Bulk quantities of the medium are available (see *Ordering information*).

Regeneration is intended to restore the original functionality of the affinity medium. Depending on the nature of the sample, the medium is normally regenerated after each purification cycle and then re-equilibrated in binding buffer. To prevent the build up of contaminants over time, more rigorous protocols may have to be applied (see *Cleaning-in-place*).

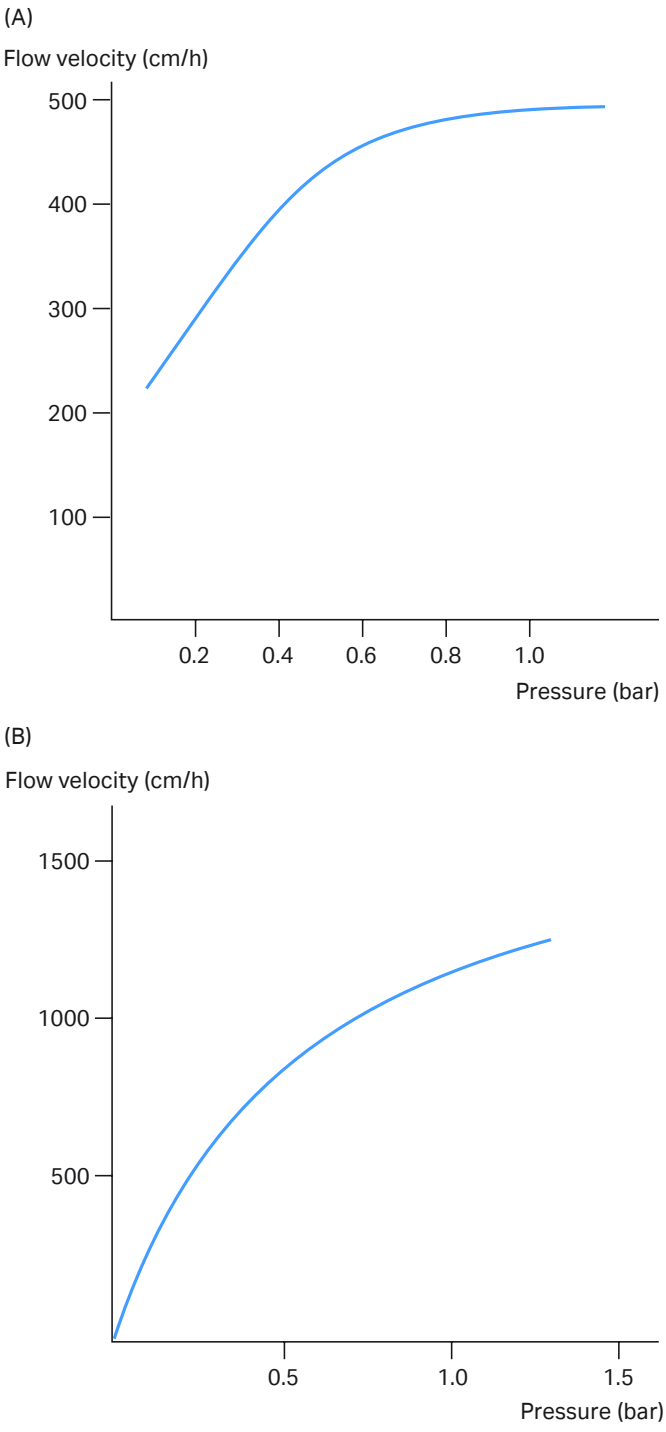

Fig 2. Pressure/flow velocity curves for Sepharose 4 Fast Flow in (A) a K 50/30 column, bed height 15 cm, (B) a BP 113 column, bed height 5 cm (Work from Cytiva).

Stability

The amide bond between the ligand and the spacer arm is stable over almost the entire pH range. However, long-term exposure above pH 12 should be avoided as ligand hydrolysis is accelerated at high pH. Note, however, that the reduction in ligand density after three months storage in 0.1 M sodium hydroxide is only 10%. Figure 3 shows more ligand density/stability data.

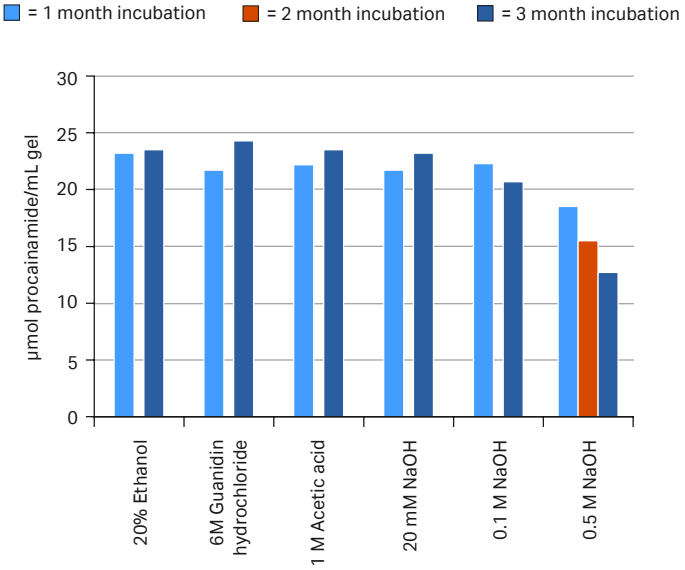

Fig 3. Ligand density of Procainamide Sepharose 4 Fast Flow after storage in different solutions at ambient temperature for 1, 2 or 3 months.

Cleaning-in-place and sanitization

A cleaning-in-place (CIP) and sanitization protocol has to be designed for each application. A general recommendation is to use a guanidine hydrochloride solution to remove precipitated or denatured substances. For hydrophobic-bound substances, a non-ionic detergent or ethanol is recommended. As Figure 3 shows, different concentrations of sodium hydroxide can also be used, but ensure that you keep the contact time as short as possible at higher concentrations.

Combinations of sodium hydroxide and 20% ethanol can be efficient for sanitization (3). Prolonged exposure to pH lower than 2 should be avoided as the matrix decomposes at low pH.

Storage

Procainamide Sepharose 4 Fast Flow is supplied as a suspension in 20% ethanol. Recommended storage conditions are 20% ethanol at ambient temperature.

References

1. Lockridge., *et al. Biochemistry* **36**, 786-795, (1997)

2. Lynch US patent 5272080

3. Sofer, G. and Hagel, L. Cleaning, sanitization and storage, in *Handbook of Process Chromatography: A Guide to optimization, scale-up and validation.* Academic Press, 188-214 (1997).

Ordering information

Product	pack size[1]	Code no.
Procainamide Sepharose 4 Fast Flow	100 mL	28-4111-01
Procainamide Sepharose 4 Fast Flow	1 liter	28-4111-03

[1] This product is part of our Custom Designed Media program. If you want to order larger quantities, please contact your local Cytiva representative.

cytiva.com/histrap

CY17842-22Jan21-DF