Plasminogen Removal Gel

CUSTOM DESIGNED MEDIA

Plasminogen Removal Gel is a medium designed for industrial removal of plasminogen and plasminogen activator, the target molecule being for example fibrinogen. It can also be used for purification of plasminogen. The medium is based on a highly cross-linked 4% agarose matrix, which enables rapid processing of large sample volumes. The ligand, trans-4-(aminomethyl) cyclohexane carboxylic acid, is attached to the base matrix via a long, hydrophilic spacer arm to make it easily available for binding of the target molecule (Fig 1).

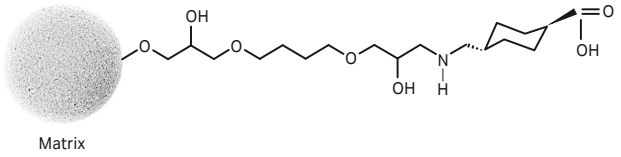

Fig 1. Partial structure of Plasminogen Removal Gel.

Characteristics

Table 1. Main characteristics of Plasminogen Removal Gel

Matrix	Macroporous, cross-linked 4% agarose
Average particle size:	90 µm
Ligand:	Trans-4-(aminomethyl)cyclohexanecarboxylic acid
Ligand density:	9–13 µmol/mL drained medium
Flow velocity:	150–250 cm/h, 25 cm bed height, 0.1 MPa, distilled water in XK 50 column
pH stability	
Long term:	3–13
Short term:	2–14

Principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them under suitable elution conditions. These conditions depend on the target molecule, feed composition, and chromatography medium, and must be studied together with other chromatographic parameters (i.e., sample load, flow velocity, bed height, regeneration, cleaning-in-place, etc.) to establish the conditions that will bind the largest amount of target molecule, in the shortest time and with the highest product recovery.

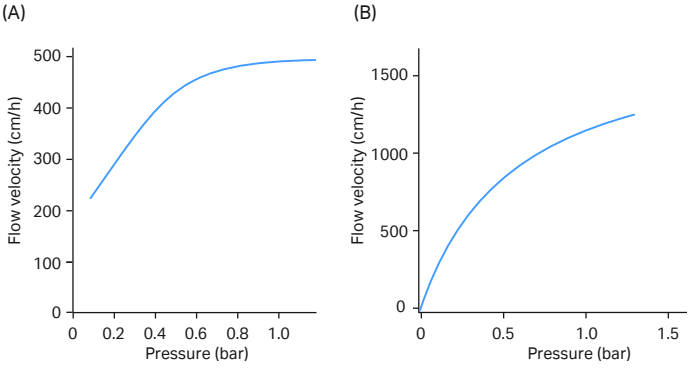

Fig 2. Pressure and flow velocity curve for Sepharose 4 Fast Flow in (A) K 50/30, bed height 15 cm, and (B) BP 113, bed height 5 cm.

We recommend a bed height of 10 to 15 cm to allow high flow rates to be used. As a guide, pressure and flow curves for the base matrix Sepharose™ 4 Fast Flow in a K 50/30 and a BP 113 column are shown in Figure 2. For example, plasminogen adsorbs to Plasminogen Removal Gel when plasma (sodium chloride is added to a final concentration of 0.1 M) is pumped through the column. The plasminogen is then eluted* with the desorption buffer (adsorption buffer + 50-mM ε-aminocaproic acid).

Regeneration should restore the original function of the medium. Depending on the nature of the sample, regeneration is normally performed after each cycle followed by re-equilibration in start buffer. To prevent build up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization below).

* Unspecifically bound proteins are washed away with a buffer (50-mM sodium dihydrogen phosphate, 0.1-M sodium chloride, pH 7.4).

Stability

The bond between the ligand and the spacer arm is stable under both acidic and alkaline conditions. In a study where Plasminogen Removal Gel was stored in various solutions under ambient conditions for nine months, insignificant change in ligand concentration was observed (Fig 3). Furthermore, the study showed no change in functional performance, measured as the plasminogen binding capacity, in the same stored samples.

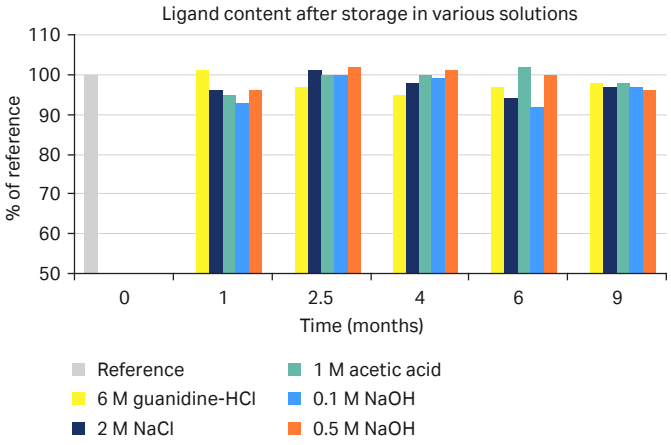

Fig 3. Ligand content of Plasminogen Removal Gel after different periods of storage under ambient conditions, as percentage of reference.

Cleaning-in-place and sanitization

A cleaning or sanitization protocol has to be designed for each application. Generally, sodium hydroxide (0.1 to 1 M), alone or in combination with sodium chloride (0.5 to 3 M) or ethanol (20% to 70%), is an effective sanitization agent. Prolonged exposure (i.e., several days) to pH < 2 should be avoided due to a slow decomposition of the matrix at low pH. Strongly bound proteins can be removed with urea or guanidine hydrochloride (1).

Storage

We recommend that the medium is stored at pH 5–7 in 20% ethanol. Plasminogen Removal Gel is supplied pre-swollen in a buffered 20% ethanol solution.

Reference

1. Sofer, G. and Hagel, L. Cleaning, Sanitization, and Storage, in *Handbook of Process Chromatography*: A Guide to Optimization, Scale-up, and Validation, Academic Press, pp. 188–214 (1997).

Ordering information

Product	Quantity	Code number
Plasminogen Removal Gel*	1 L	28-4109-03

* This product is part of our Custom Designed Media program and not yet a standard product. If you are interested in large-scale quantities, please contact your local Cytiva representative.

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