

## Instructions for TSKgel Heparin-5PW

### Support

Heparin-5PW was prepared by introducing heparin into G5000PW.

Amount of immobilized ligand : 4 - 6 mg/ml

Particle size : 10  $\mu$ m

Pore size : ca. 1000 Å

### Column

Size : 75 x 7.5 mm I.D.

Solvent : distilled water

### pH range for separation

5 - 10

### Salt concentration range

less than 3 M

### Flow rate range

less than 1.2 ml/min

Flow rates of 0.5 - 1.0 ml/min are recommended in general.

### Column washing

0.1 - 0.2 N NaOH are very effective to wash or regenerate columns.

Usually, columns can be regenerated by injecting 0.1 - 0.2 N NaOH of 1 - 2 ml several times using sample injector. When this procedure did not help, wash the column by injecting 20 - 40 % acetic acid of 1 - 2 ml several times.

### Guard column

The use of guard column (10 x 6 mm I.D.) is recommended for a long service life of analytical columns.

The change of both inlet side filter and support once a week is recommended for every day use.

The guard column can be packed by pouring the concentrated slurry of Heparin-5PW guard gel into it and then sucking with aspirator.

The old filter can be re-used after washing by exposing it to ultrasonic in 0.1 - 0.2 N NaOH for about 30 min.

### Heparin-5PW guard gel

Support prepared by introducing heparin into G5000PW of 20 - 30  $\mu$ m in particle diameter for use in guard column

### Storage

When Heparin-5PW columns are stored for more than several days, replace the solvent in columns with distilled water.

Store the columns at around 4°C.

### Test for theoretical plate number

Following conditions are recommended to test columns for their theoretical plate numbers and As (asymmetry factor).

Eluent : 10 mM sodium acetate buffer (pH 5.0)\*

Flow rate : 1.0 ml/min

Sample : cytidine (0.05 %, 20  $\mu$ l)

Heparin-5PW columns have theoretical plate numbers more than 1,300 plates per column and As of 0.8 - 1.6 at a time of delivery.

\* Prepare 10 mM sodium acetate aqueous solution and 10 mM acetic acid aqueous solution.

Add the two aqueous solutions properly so that the pH of the mixture becomes 5.0.