GAG Binding Plate

According to customer feedback this protocol works slightly better than the original. Also using an HRP-conjugated antibody as the detecting system works better and has a lower background.

#Cat No. : H/G Plates

Example of an experimental protocol for investigation of protein binding to heparin immobilized on the GAG Binding Plate: Interaction of IL-8 with Heparin

Reagents and Solutions

1) Standard Assay Buffer (SAB):

100mM NaCl, 50mM sodium acetate, 0.2% v/v Tween 20 pH7.2

2) Heparin (Iduron Inc) Solution:

1 μg/mL of Heparin in SAB

3) Blocking Solution:

1% w/v BSA /PBS (0.2% (w/v) gelatin in SAB (gelatin from fish skin, Sigma product) has also been used as an effective blocking agent)

4) Human IL-8 (Peprotech Inc):

0.8 μg/mL in Blocking Solution

5) Biotinylated Anti-Human IL-8 (Peprotech Inc):

250 ng/mL in Blocking Solution

6) HRP-Streptavidin (Merck Millipore Inc):

100 ng/mL in Blocking Solution

7) Peroxidase Assay Kit for ELISA(Sumitomo Bakelite Inc)

Procedure for Coating Heparin/GAG Binding Plates with Heparin

1) Add 200ul of Heparin Solution per well

2) Incubate overnight at room temperature protected from light

3) Unwrap plates and carefully decant supernatant to waste.

4) Wash plates carefully three times with SAB

5) Add 250 μl of Blocking Solution per well

6) Incubate at 37˚C for 1 hour protected from light

7) Wash plate carefully three times with SAB, tap to remove residual liquid

– do not allow the plate to dry at this or any step in the assay.

At this stage the plate is ready for use and ideally it is best to use it immediately.

If necessary plates can be stored at 4˚C overnight, sealed to avoid evaporation and protected from light.

Assay of Protein Binding to Immobilised Heparin

The following procedure has been used to study the binding of the chemokine IL-8 to heparin. It is provided as an indication of the conditions that might be used for other assays. Preliminary experiments should be carried out to test the suitability of these conditions for other heparin/GAG-binding proteins and for other applications.

1) Dissolve human IL-8 in Blocking Solution at a concentration of 0.8 μg/mL
2) Dispense 100 μl of each dilution of IL-8 into wells of GAG Binding Plates coated with heparin.
3) Incubate for 1hr at 37˚C
5) Wash carefully three times with SAB
6) Add 100ul of 250 ng/mL biotinylated anti-Human IL-8 in Blocking Solution
7) Incubate for 1hr at 37˚C
8) Wash carefully three times with SAB
9) Add 100 μl of 100ng/mL HRP-Streptavidin in Blocking Solution
10) Incubate for 1hr at room temperature
11) Wash carefully three times with 0.1% v/v Tween 20 /PBS and tap to remove residual liquid
12) Use the Peroxidase Assay Kit
13) Read at 450nm_

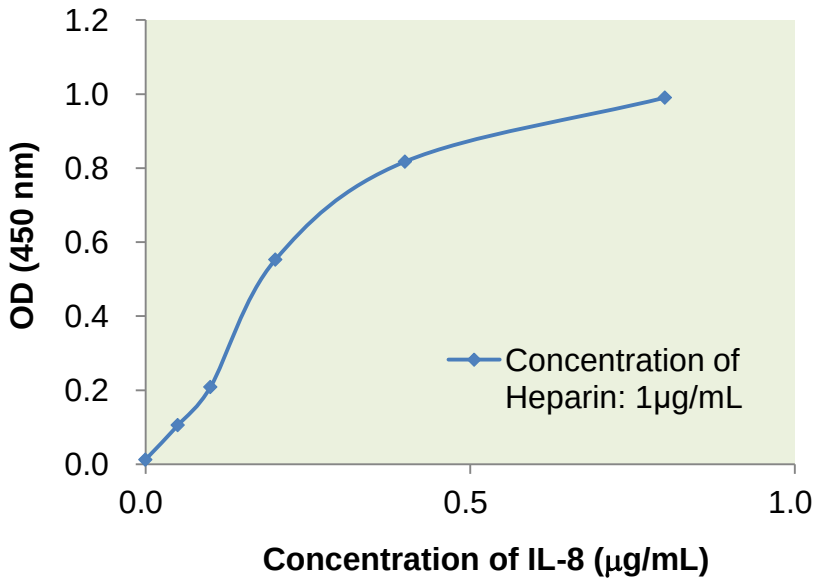

Fig.1 IL-8 binding capacity of heparin preparations coated on Heparin/GAG Binding Plate