

Introduction

The glycosaminoglycan (GAG) heparin is a linear polysaccharide consisting of repeating disaccharide units. These units contain L-iduronic acid or its epimer D-glucuronic acid or D-glucosamine, which is either N-acetylated or N-sulfated. Heparin is a highly sulfated form of heparan sulfate (HS), a ubiquitous molecule of the extracellular matrix which is covalently attached to proteins called proteoglycans. These molecules are involved in a wide range of fundamental biological processes, including proliferation, differentiation, tissue homeostasis, and viral pathogenesis. This multiplicity of function arises through sequence diversity within the HS chain. Although structurally diverse, heparin is an excellent model for studying HS-protein interactions because it is very similar in structure to the sulphated regions of HS and more readily available.

This glycosaminoglycan is unable to adhere to the surface of conventional polystyrene microplates. As a result, the development of high throughput ELISA-like assays using surface immobilized heparin had been hindered. The BD™ Heparin Binding Plate has been modified to produce a surface to which heparin can be immobilized. Heparin immobilized onto the plate surface is able to interact with heparin-binding proteins¹. In addition, a range of heparin preparations ranging in size from high molecular weight to a defined decasaccharide can be adsorbed onto BD Heparin Binding Plate in a functionally active form. The availability of the Heparin Binding Plate facilitates the identification and characterization of heparin/HS-binding proteins and enables a wide range of applications in many areas of biology.

Materials and Methods

Depending on the compound to be targeted, preparation of the BD Heparin Binding Plate requires coating of the plate with the desired heparin solution.

Heparin Binding

All materials were obtained from Sigma-Aldrich.

- Phosphate Buffered Saline (pH 7.4, 0.01 M)
- Acetate Buffer (100 mM NaCl, 50 mM NaAc, 0.2% Tween-20 pH 7.2)
- Low Molecular Weight (LMW) heparin
- Gelatin Blocking Solution (0.2%)

LMW heparin was dissolved in PBS at 25 µg/ml and 200 µl of heparin was added to each well and incubated on a BD Heparin Binding Plate overnight at room temperature.

Note: The dispensed volume can be adjusted depending upon the chosen format of subsequent assay steps—for example, with valuable reagents it might be desirable to reduce the assay volume to 100 µl or less.

After overnight incubation, the supernatant was discarded. The BD Heparin Binding Plate was washed with acetate buffer and blocked with gelatin blocking solution for 1 hour at 37°C, followed by washing with acetate buffer.

Detection of Bound Heparin using IL-8

Following the binding of heparin to a BD Heparin Binding Plate, IL-8 (a heparin binding protein) was used to detect functionally active heparin on the plate surface. The assay principle is represented in **Figure 1**. All antibodies were obtained from Peprotech Inc. and reagents were obtained from Sigma-Aldrich.

- Acetate Buffer (100 mM NaCl, 50 mM NaAc, 0.2% Tween-20 pH 7.2)
- Recombinant Human IL-8
- Biotinylated Anti-Human IL-8
- ExtrAvidin-AP (Alkaline Phosphatase)
- Sigma FAST™ pNPP tablets
- Gelatin Blocking Solution

Human IL-8 was incubated at (0-5 µg/ml) for 2 hours at 37°C. The plate was then washed and incubated with 250 ng/ml anti-human IL-8 for 1 hour at 37°C. After washing with buffer 220 ng/ml ExtrAvidin was added and incubated for 30 minutes at 37°C. The BD Heparin Binding Plate was then washed with buffer and developed with pNPP for 40 minutes before measuring the absorption at 405nm.

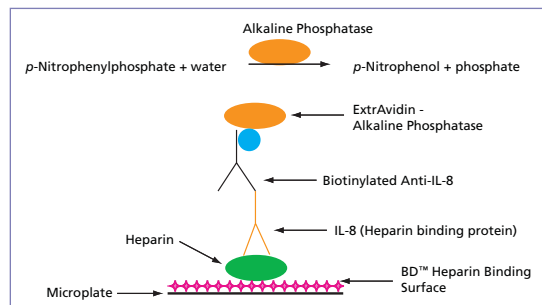


Figure 1: Depiction of BD™ Heparin Binding Plate used for detection of IL-8 binding heparin.

Results and Discussion

Numerous heparin and heparan sulfate preparations were successfully immobilized onto the solid support of the BD™ Heparin Binding Plate. Three such preparations are illustrated in Figure 2. Heparin fractions bound to the plate were assayed to measure retained functionality. The BD Heparin Binding Plate successfully bound each heparin preparation to the surface in a functionally active form. While using IL-8 heparin binding protein, µg concentrations successfully bound to the immobilized heparin for robust detection. IL-8 is one of many heparin-binding proteins that can be used in conjunction with the BD Heparin Binding Plate. Table 1 compiles GAGs functionally evaluated on the BD Heparin Binding Plate to date.

Summary

- Immobilize functionally active heparin
- Wide range of heparin preparations can be immobilized based on desired protein or compound being studied
- Microplate format allows high throughput
- Less sample required than current methodologies (µg versus mg)
- Improved signal to noise over current methodologies

Reference

1. D.J. Mahoney, J.D. Whittle, C.M. Milner, S.J. Clark, B. Mulloy, D.J. Buttle, G.C. Jones, A.J. Day, R.D. Short, A method for the non-covalent immobilization of heparin to surfaces, *Analytical Biochemistry* **330**:123 (2004).

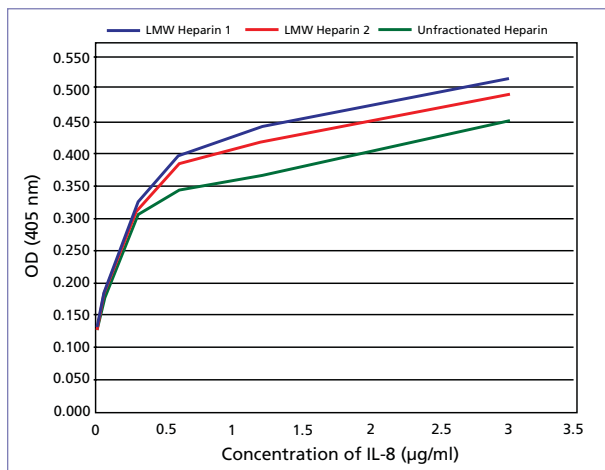


Figure 2: Comparison of three heparin preparations used to coat wells of a BD™ Heparin Binding Plate at 25 µg/mL, which is saturating for heparin binding. All three show similar response curve up to about 0.3 µg/mL IL-8 but differ in the signal at saturation, indicating differences between the heparins in terms of IL-8 binding capacity.

Glycosaminoglycans Evaluated Successfully

- Low Molecular Weight Heparin
- High Molecular Weight Heparin
- Heparan Sulfate
- Dermatan Sulphate
- Chondroitin Sulphate
- Therapeutic LMW heparins
 - Enoxaparin (known as Lovenox or Clexane)
 - Dalteparin (known as Fragmin)
- Modified Heparin Fractions
 - 2-O-desulphated
 - 6-O-desulphated
 - 2,6-O-desulphated
 - N-desulphated
 - re-N-acetylated

Table 1: Table 1: GAGs successfully bound to the BD™ Heparin Binding Plate to date. This list is non-inclusive, as additional preparations are theorized to bind equally well.

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