

# Plasso EpranEx™ ready to use heparin-binding plate with the FLUOstar OPTIMA plate reader



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- Immobilises functionally active heparin
- Wide range of heparin preparations can be used
- Microplate format allows high throughput
- Assay can be used with the FLUOstar OPTIMA, versatile reader for luminescence, absorbance and fluorescence

## Introduction

The glycosaminoglycan (GAG) heparin is a linear polysaccharide consisting of repeating disaccharide units containing L-iduronic acid (or its epimer D-glucuronic acid) and D-glucosamine (which is either N-acetylated or N-sulfated). It is one of the best-known mast cell-derived immune mediators, and also acts as an anti-coagulant through its activation of serine protease inhibitors (serpins).

Heparin is a highly sulfated form of heparan sulfate (HS), a ubiquitous molecule of the extracellular matrix covalently attached to proteins (proteoglycans) and involved in a wide range of fundamental biological processes including cellular proliferation, differentiation, tissue homeostasis and viral pathogenesis. This multiplicity of function arises through sequence diversity within the HS chain. 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, although still structurally diverse.

The development of high throughput ELISA-like assays using surface immobilized heparin has been hindered by the inability of this glycosaminoglycan to adhere to the surface of conventional polystyrene microplates. The Plasso EpranEx™ plate has been modified to produce a surface onto which heparin can be immobilised. Heparin immobilised onto the plate surface is able to interact with heparin-binding proteins [1]. In addition a range of heparin preparations ranging in size from high molecular weight to a defined decasaccharide can be adsorbed onto EpranEx™ heparin-binding plates in a functionally active form. The availability of the Plasso EpranEx™ plate facilitates the identification and characterisation of heparin/HS-binding proteins and enables a wide range of applications in many areas of biology.



Fig. 1: FLUOstar OPTIMA

## Materials and Methods

### 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)
- LMW heparin (low molecular weight)
- Gelatin Blocking Solution (0.2%)

LMW heparin was dissolved in PBS at 25ug/ml and incubated on an EpranEx™ plate overnight at room temperature. The EpranEx™ plate was washed with acetate buffer and blocked with gelatin blocking solution for 1 hour at 37C, followed by washing with acetate buffer. Detailed protocols and advice can be found in the EpranEx™ booklet or at [www.plasso.com](http://www.plasso.com).

### Detection of Bound Heparin using IL8

Following the binding of heparin to an EpranEx™ plate, IL8 (a heparin binding protein) was used to detect functionally active heparin on the plate surface. The assay principle is give in Fig. 2. All antibodies were obtained from Peprotech Inc. and reagents were obtained from Sigma-Aldrich.

- acetate buffer (100mM NaCl, 50mM NaAc, 0.2% Tween-20 pH 7.2)
- recombinant human IL8
- biotinylated anti-human IL8
- ExtrAvidin-AP (alkaline phosphatase)
- Sigma FAST™ pNPP tablets
- Gelatin Blocking Solution

Human IL8 was incubated at (0-5ug/ml) for 2 hours at 37C. The plate was then washed and incubated with 250ng/ml anti-human IL8 for 1 hour at 37C. After washing with buffer 220ng/ml ExtrAvidin was added and incubated for 30 minutes at 37C. The EpranEx™ plate was then washed with buffer and developed with pNPP for 40 minutes before measuring the absorption at 405nm on the FLUOstar OPTIMA plate reader. Detailed protocols and advice can be found in the EpranEx™ booklet or at [www.plasso.com](http://www.plasso.com)

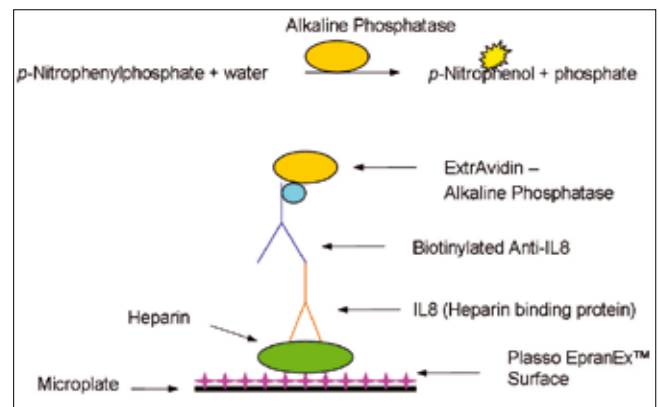


Fig. 2: Assay principle

## Results and Discussion

Data were evaluated using Microsoft Excel™ in conjunction with BMG LABTECH FLUOstar Excel™ evaluation package.



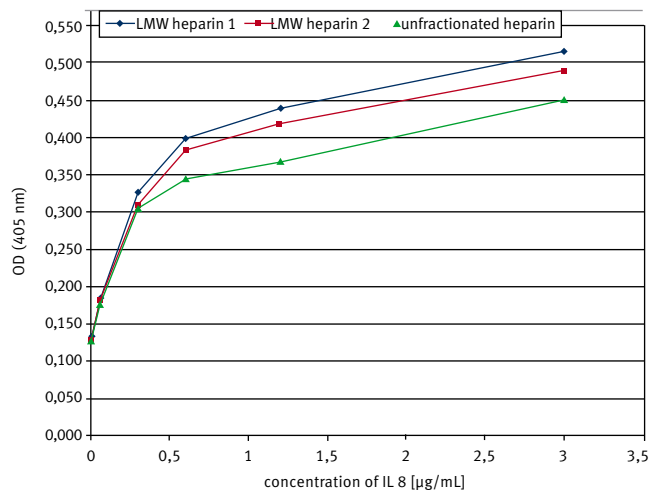
**Fig. 3:** Test settings for detection of heparin using IL8

A point to point linear regression curve was used for analysis, with each concentration averaged from triplicate samples (figure 4).

The EpranEx™ plate successfully bound LMW heparin to the surface in a functionally active, immobilised form.

IL8 is just one of many heparin-binding proteins that can be used in conjunction with the EpranEx™ plate and the BMG FLUOstar plate reader. The BMG FLUOstar plate reader can also detect fluorescence and luminescence allowing flexibility in detection methods, if required.

EpranEx™ is a trade mark of Plasso Technology Ltd and is patent protected.



**Fig. 4:** Comparison of three different heparin preparations using IL-8 binding following the Binding Assay protocol above. All three heparin preparations were used to coat wells of an EpranEx™ plate at 25 µg/mL, which is saturating for heparin binding. All three show the same response curve up to about 0.3µg/mL IL-8 but differ in the signal at saturation, indicating differences between the heparins in terms of IL-8 binding capacity

## References

- [1] D] Mahoney et al. 'A method for the non-covalent immobilisation of heparin to surfaces'. Analytical Biochemistry Vol. 330(2004) p123-129.

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