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Iduron technical bulletin - Heparin/GAG Binding Plates

Protocols for use with Iduron Heparin/GAG Binding Plate

For research use only. Not for use in humans.
Not for clinical, diagnostic or therapeutic applications.



Heparin/GAG Binding Plates from Iduron

- Surface immobilisation of native glycosaminoglycans in a 96-well format.
- Retention of protein binding properties.
- Versatile, simple to use, amenable to high throughput.

Introduction

Investigations of the protein-binding properties of heparin, heparan sulfate (HS) and other glycosaminoglycans (GAGS) have been constrained by the need for chemical modification prior to attachment to inert or derivatised surfaces. The Iduron Heparin/GAG Binding Plates offer a simple solution to this problem. The specially-prepared plate surface adsorbs GAGS without modification whilst retaining their protein-binding characteristics. Binding occurs at room temperature from physiological buffers. The versatility of the Iduron Heparin/GAG Binding Plates will find many applications in basic and applied studies of protein interactions with GAGS including elucidation of optimum GAG sequences and sulphation patterns for binding to proteins of interest, analyses of sulfotransferases and endosulfatases and detection of inhibitors or enhancers of protein-GAG recognition. The plates can also be used for studying receptor binding to growth factors or chemokines bound to surface-immobilised GAGS. The ELISA-type format is economical on the use of valuable protein reagents and is compatible with automated laboratory systems.

Iduron has designed GAG Arrays for use in conjunction with these plates - see later

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

Reagents and Solutions

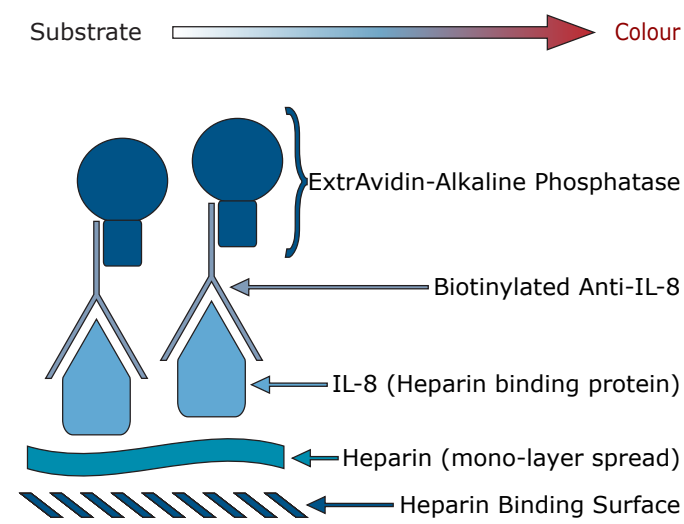
- 1) Standard Assay Buffer (SAB):
100mM NaCl, 50mM sodium acetate, 0.2% v/v Tween 20 pH7.2
- 2) Heparin Solution:
25ug/ml of Heparin in SAB
(Iduron's High Grade Heparin or Low in Calcium Heparin are recommended)
Optimum concentrations for other GAGS can be determined by coating with a dilution range from 0-50ug/ml.
- 3) Blocking Solution:
0.2% (w/v) gelatin in SAB (gelatin from fish skin, Sigma product)
(1% w/v BSA in PBS has also been used as an effective blocking agent)
- 4) Human IL-8 (Peprotech Inc):
3ug/ml in Blocking Solution
- 5) Biotinylated Anti-Human IL-8 (Peprotech Inc):
250ng/ml in Blocking Solution
- 6) ExtrAvidin AP (Sigma):
220ng/ml in Blocking Solution
- 7) Development Reagent (Sigma):
Sigma FAST p-Nitrophenyl phosphate tablets
Prepare according to manufacturers instructions.

Recommended 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 250ul 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.

Detection of IL8 bound to Heparin

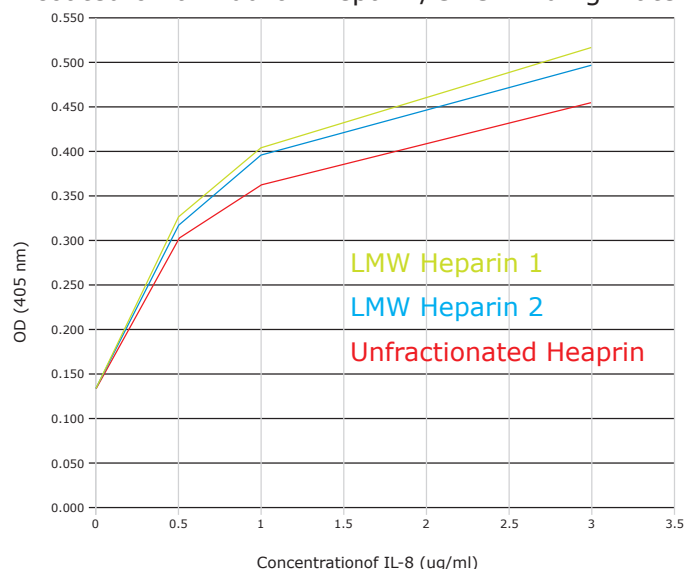


Assay of Protein Binding to Immobilised Heparin

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 (0.2% gelatin in SAB) at a concentration of 3ug/ml
- 2) Generate a dilution series from 0-3ug/ml in Blocking Solution
- 3) Dispense 200ul of each dilution of IL-8 into triplicate wells of Heparin/GAG Binding Plates pre-coated with heparin.
- 4) Incubate for 2hrs at 37°C
- 5) Wash carefully three times with SAB
- 6) Add 200ul of 250ng/ml biotinylated anti-Human IL-8 in Blocking Solution
- 7) Incubate for one hour at 37°C
- 8) Wash carefully three times with SAB
- 9) Add 200ul of 220ng/ml ExtrAvidin-AP in Blocking Solution
- 10) Incubate for 30mins at 37°C
- 11) Wash carefully three times with SAB and tap to remove residual liquid
- 12) Add 200ul of Development Reagent – SigmaFAST p-Nitrophenyl phosphate
- 13) Incubate at room temperature for 40 minutes
- 14) Read at 405nm within one hour

IL8 binding capacity of three heparin preparations coated on an Iduron Heparin/GAG Binding Plate



Glycosaminoglycans and Derivatives Successfully Evaluated

- a) High Molecular Weight Heparin (cat. no. HEP001)
- b) Low Molecular Weight Heparin (cat. no. HO30)
- c) Heparin Oligomers down to Decasaccharide (cat. no. HO10 - HO26)
- d) Heparan Sulfate (cat. no. GAG-HS01)
- e) Dermatan Sulfate (cat no. GAG-DS01)
- f) Chondroitin Sulfate
- g) Hyaluronan (cat. no. HA01)
- h) Chemically-Modified Heparins:
 - 2-O-desulfated (cat. no. DSH001/2)
 - 6-O-desulfated (cat. no. DSH002/6)
 - 2-O,6-O desulfated
 - N-desulfated/reN-acetylated (cat. no. DSH004pNAc)

Heparin/HS Binding Proteins

FGF1, FGF2, FGF7 and FGF10	Members of the FGF family of growth factors and morphogenic proteins
HGF/SF	Hepatocyte Growth Factor/Scatter Factor
IL-8	Interleukin 8
KC	Mouse Chemokine KC
PDGF	Platelet Derived Growth Factor
PIGF	Placenta Growth Factor
VEGF	Vascular Endothelial Growth Factor

The above proteins have been demonstrated to bind to heparin coated on the Heparin/GAG Binding Plates. For any particular protein it is important to determine the most suitable glycosaminoglycan and assay procedure to optimise the conditions of interaction with the target protein. Iduron cannot warrant that all heparin-binding proteins will bind to GAGS immobilised on the plates.

More Technical Bulletins from Iduron:
 GAG Arrays
 Sulphated K5 Polysaccharides



Alternative Protocols

Phosphate buffered saline (PBS) can be used as an alternative standard assay buffer (SAB) and 1% w/v BSA in PBS has been shown to be an effective alternative blocking agent to 0.2% gelatin.

In the above example IL-8 was dissolved in Blocking Solution (SAB containing 0.2% gelatin) and likewise the detection antibody and the ExtrAvidin-AP were dissolved in this same blocking solution with the gelatin present. This was done to ensure that the surface of the wells remained blocked with gelatin throughout the binding and detection stages. In practice this may not be necessary and it may only be required to incubate the plates with the blocking protein once at step 5 in Recommended Procedure for Coating Heparin/GAG Binding Plates with Heparin i.e. the first step after binding the heparin to the plate surface. It is possible that in some circumstances the blocking protein could interfere with binding of the experimental protein to the heparin or it could affect the antibody detection system. We recommend that preliminary experiments are carried out to determine the most effective blocking procedure compatible with low background signal and specific protein interaction with the bound heparin.

Other procedures could be used for detecting bound proteins. For example the specificities of cognate signaling receptors could be exploited to identify growth factors (or chemokines) bound to GAGS immobilised on the plate surface; alkaline phosphatase (AP)-receptor fusion proteins or specific anti-receptor antibodies could be used for detection and quantitation. In this way structural requirements for GAGS to mediate specific protein-protein interactions involved in cell growth regulation or cell migration could be investigated. This experimental set-up has applications for screening potential inhibitors of receptor binding to ligand-GAG complexes.

GAG-Arrays

Iduron GAG-Arrays can be used in conjunction with the plates to investigate the selectivity and plasticity of GAG-protein interactions and to find the most suitable GAG species for efficient binding to the protein of interest.

A Technical Bulletin on GAG Arrays and a Technical Bulletin on our Sulphated K5 Polysaccharides can be downloaded from our website; info@iduron.co.uk or requested by email info@iduron.co.uk.

Product Specification

Microplate Type: Clear 96-well configuration complies with ANSI/SBS standards.

5 individually packaged plates per pack

Stable for at least 6 months when stored dry at 4 - 30°C

Quality Control: Uniform binding distribution of heparin to each well via labelled bioassay.

