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Product No: S-6000
Heparan Sulfate ELISA for Plasma
Liquid Stable Conjugate
Range 0.1 – 30µg/ml

S-6000: Heparan Sulfate (HS) ELISA for Plasma Samples

INTENDED USE: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT INTENDED FOR CLINICAL OR DIAGNOSTIC USE.

Kit includes:

Coated 96-well plate
Detector -Enzyme Conjugate (stabilized liquid)
TMB Solution
Stop Solution
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)

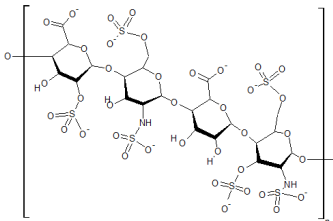
Researcher must provide:

Pipettes (8 Channel Multipipettor is recommended)
Absorbance microplate reader
Heparan Sulfate standards from your heparan sulfate
Standard Diluent Normal Plasma (platelet depleted)
Plate Cover

Storage and Stability

Kit can be stored unopened at 4°C for up to six months. The Detector-Enzyme Conjugate Solution and the TMB solution should be protected from light.

Background



Heparan sulfate (HS) is a glycosaminoglycan that is found on the surface of most cells as part of proteoglycans. Heparan sulfate is also present in the extracellular matrix. The polysaccharide mediates the interactions between a number of different proteins.

The HS ELISA, product number S-6000, is a quantitative enzyme-linked assay designed for the *in vitro* measurement of HS levels in plasma. This assay measures HS directly using a HS binding protein which has been conjugated to HRP.

The HS ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of HS present in the sample. Samples to be assayed are first mixed with the detector-enzyme conjugate in wells of the coated plate. HS in the sample competes with HS bound to the plate for binding of the detector-enzyme conjugate. The concentration of HS in the sample is determined using a standard curve of known amounts of HS.

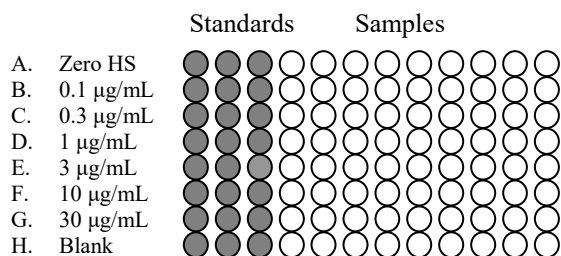
Crossreactivity

Other endogenous glycosaminoglycans such as hyaluronic acid, keratan sulfate and chondroitin sulfate cross-react less than 1%.

Reagent Preparation

Heparan Sulfate Standards: Prepare standards using fresh normal human plasma that has been platelet depleted and your Heparan Sulfate to obtain standards of 0.1, 0.3, 1, 3, 10, and 30µg/mL. **Standardization should be performed using Heparan Sulfate that is the same Heparan Sulfate type contained in your unknowns.**

1X Wash Buffer: Make a 1:10 dilution of 10X Wash Buffer in distilled or deionized water.



Heparan Sulfate ELISA Plate

Assay Procedure

1. Set up the Heparan Sulfate ELISA plate as illustrated above. We suggest the Heparan Sulfate standard dilution series be run in triplicate for best results. Add **10 µL** of standards and samples into corresponding wells. Add **90 µL** of Detector -Enzyme Conjugate to all wells except the blank wells. Mix well. Cover plate and incubate for one hour at room temperature. A rotator is highly recommended, if available, as constant mixing significantly improves precision.
2. Discard the solution and wash the wells four times with 300 µL per well of 1X Wash Buffer. An automated plate washer is recommended if available. After washing, immediately proceed to the next step. Do not delay in removing wash buffer from the wells. Do not allow plate to dry.
3. Add 100 µL TMB Solution to each well. Incubate the plate in the dark at room temperature for 4-60 minutes waiting for the zero Heparan Sulfate wells to develop to a medium to dark blue color. Watch for color development and **DO NOT** overdevelop.
4. Add 50 µL Stop solution, which will change the color from blue to yellow.
5. Immediately measure the absorbance of each well at 450 nm.
6. Calculate the binding percentage for each sample using the formula:

$$[A_{450}(\text{Sample}) - A_{450}(\text{Blank})] / [A_{450}(\text{Zero Heparan Sulfate}) - A_{450}(\text{Blank})] \times 100 = \% \text{ Binding}$$

Using linear or nonlinear regression, plot a standard curve of percent binding versus concentration of Heparan Sulfate standards. Determine Heparan Sulfate levels of unknowns by comparing their percentage of binding relative to the standard curve. Heparan Sulfate can be estimated by comparing the values from the wells containing unknowns to the values in the standard curve.

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