**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.
**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:

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\frac{[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.