

Produktinformation



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Zellkultur & Verbrauchsmaterial
Diagnostik & molekulare Diagnostik
Laborgeräte & Service

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Lieferung & Zahlungsart siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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- Expressversand

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Lifespan Technologies PO Box 3352 Salt Lake City, UT 84110 Tel: 801-971-1818 Fax: 801-464-6116 info@lifespantech.com 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

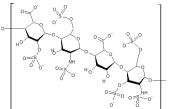
INTENDED USE: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT INTENDED FOR CLINICAL OR DIAGNOSTIC USE.

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 readerHeparan Sulfate standards from your heparan sulfateStandard Diluent Normal Plasma (platelet depleted)Plate Cover
wash Concentrate TOX, (dilute 1 part plus 9 parts water)	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



^L 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.

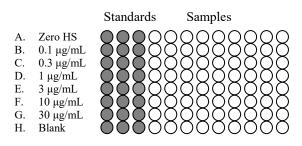
Crossreactivity

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

Reagent Preparation

<u>Heparan Sulfate Standards</u>: 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.

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



Assay Procedure

Heparan Sulfate ELISA Plate

- 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.
- 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.
- 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:

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

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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