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

Product No: **S-4000**
LMW Dextran Sulfate ELISA
For Plasma or High Protein Samples
Liquid Stable Conjugate
Range 0.3 – 100ug/ml

S4000: Low Molecular Weight Dextran Sulfate ELISA for Plasma or High Protein

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

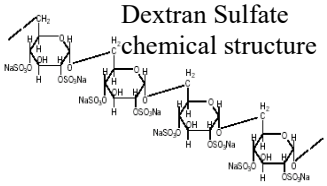
Kit includes:
Coated 96-well plate
Detector -Enzyme Conjugate (stabilized liquid)
TMB Solution
Stop Solution
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)
Pretreatment for High Protein Samples (Ready to Use)

Researcher must provide:
Pipettes (8-channel multi-pipettor recommended)
Absorbance microplate reader
Low Molecular Weight Dextran sulfate standards
Fresh plasma or other high protein medium
Plate Sealer

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


Dextran Sulfate chemical structure

Dextran Sulfate is in the family glycosaminoglycan. It is a polyanionic dextran derivative which may be synthesized from various high purity and well-characterized dextran fractions. In clinical research anticoagulant dextran sulfate properties have been tested as a possible substitute for heparin in anticoagulant therapy. Another source of interest relates to the effect of dextran sulfate on enzyme inhibition in certain biological systems. Dextran sulfate is used to precipitate LDL and VLDL in plasma fractionation procedures. Dextran sulfate must then be removed from the product. The S-4000 assay allows measurement of dextran sulfate and gives manufacturers quantitative data that they have added the appropriate level or have subsequently removed dextran sulfate from their product.

The dextran sulfate ELISA product number S-4000 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of low molecular weight dextran sulfate levels in plasma or other high protein mediums. This assay measures dextran sulfate directly using a dextran sulfate binding protein which has been conjugated to HRP.

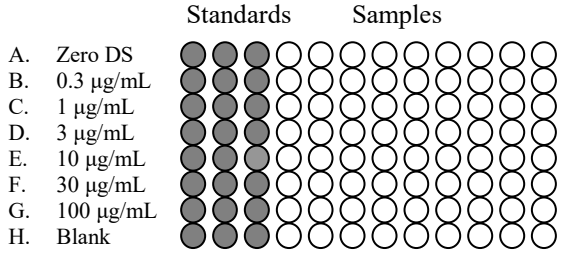
The dextran sulfate-ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of dextran sulfate present in the sample. Samples to be assayed are first mixed with the detector-enzyme conjugate in wells of the coated plate. Dextran sulfate in the sample competes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

Reagent and Sample Preparation

Dextran sulfate Standards: Prepare standards using your dextran sulfate and the same medium as your samples are prepared in to obtain standards of 0.3, 1, 3, 10, 30 and 100 µg/mL. **Standardization should be performed using dextran sulfate that is the same dextran sulfate type contained in your unknowns.** Pretreatment of the proteinaceous standards is recommended. You should pretreat your standards and your proteinaceous samples as well. Add 1 part of each of the proteinaceous standards to 9 parts of Pretreatment Solution. Vortex thoroughly.

Sample Preparation: Pretreatment of the proteinaceous samples is recommended. Add 1 part of the proteinaceous sample to 9 parts of Pretreatment Solution. Vortex thoroughly.

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



Dextran Sulfate ELISA

Assay Procedure

1. Set up the Dextran Sulfate ELISA plate as illustrated above. We suggest the Dextran Sulfate standard dilution series be run in triplicate for best results. Add **50 µL** of Pretreated Standards and Pretreated Samples into corresponding wells. Immediately add **50 µL** of Detector Enzyme Conjugate to all wells except the Blank wells. Mix well. Cover plate and incubate for 30 minutes at room temperature. A plate rotator is highly recommended for incubations, if available, as constant mixing will significantly improve 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 Dextran 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:

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

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

7. Multiply the result by the dilution factor.