Lifespan Technologies
PO Box 711297
Salt Lake City, UT 84171
Tel: 801-971-2020 Fax: 801-464-6116
info@lifespantech.com

Product No: **K-3800**

Dextran Sulfate 5000 0.003-0.1 ug/mL
ELISA Buffer/Urine

Dextran Sulfate 5000 ELISA Kit for Buffer/Urine Samples

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

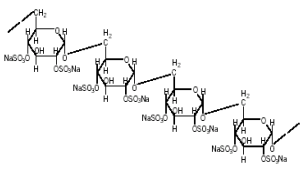
Kit includes:
Coated 96-well plate
Detector -Enzyme Conjugate vial
Conjugate Diluent
TMB Solution
Stop Solution, 0.5M H_2SO_4
Wash Concentrate 10X, (dilute 1 part plus 9 parts water to make TBS plus 0.05% TWEEN 20)

Researcher must provide:
Pipettes
Absorbance microplate reader
Dextran sulfate standard
Normal Saline (154mM NaCl)
Plate Cover

Storage and Stability

Kit can be stored unopened at 4°C for up to six months. Opened solutions can be used for up to one week when stored at 4˚C. All components and solutions should be protected from light. Reconstituted detector enzyme conjugate should be used immediately or aliquotted and stored at -80°C.

Background



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 often be removed from the product. The K-3800 assay allows measurement of extremely low levels of dextran sulfate and gives manufacturers quantitative data that they have removed dextran sulfate from their product.

The dextran sulfate ELISA product number K-3800 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of dextran sulfate levels in low protein content fluids such as buffer or urine. This assay measures dextran sulfate directly using a dextran sulfate binding protein which has been conjugated to HRP.

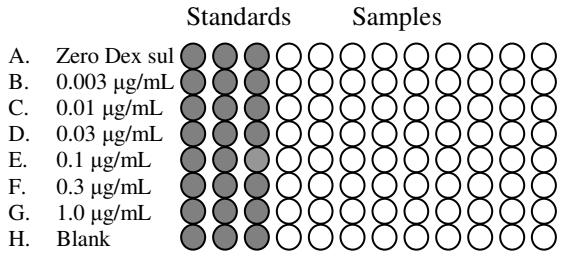
The dextran sulfate-ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of dextran sulfate present in the sample. Samples to be assayed are first mixed with the detector-enzyme conjugate in wells of the coated plate. Dextran sulfate in the sample competes 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 Preparation

Dextran sulfate Standards: Make dilutions of the appropriate dextran sulfate standard using normal saline(154mM NaCl) to obtain standards of 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 µg/mL. Standardization should be performed using dextran sulfate that is the same dextran sulfate type contained in your unknowns.

Working Detector-Enzyme Conjugate: Measure exactly **9.1 mL** of conjugate diluent and add to a clean tube. Perform a 'clean transfer' of the lyophilized Detector-Enzyme Conjugate into the 9.1 mL of conjugate diluent. This can be done by adding 500 microliters of the measured Diluent to reconstitute the Detector-Enzyme. Wait a minute to allow the lyophilized material to dissolve and then add to the liquid back to the tube. Repeat this step two more times to be sure all the Detector-Enzyme Conjugate has been transferred from the vial to the tube.

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 Standards and samples into corresponding wells. Add **50 µL** of Working 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.

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 20-60 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.

Lifespan Technologies products are sold for research and development purposes only and are not for diagnostic use or to be incorporated into products for resale without written permission form Lifespan Technologies. Materials in this publication, as well as applications, methods and use, may be covered by one or more U.S. or foreign patents or patents pending. We welcome inquiries about licensing the use of our trademarks and technologies at info@lifespantech.com.