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Product No: **K-4100**

Dextran Sulfate ELISA
for Plasma samples

K-4100: High Molecular Dextran Sulfate 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 vial
Conjugate Diluent PL
TMB Solution
Stop Solution
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)

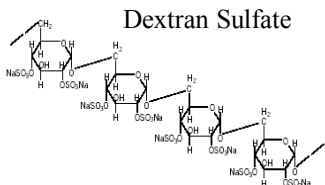
Researcher must provide:

Pipettes (8-channel multipipettor recommended)
Absorbance microplate reader
Dextran sulfate standards
Fresh normal human plasma (platelet depleted)
Plate Cover

Storage and Stability

Kit can be stored unopened at 4°C for up to six months. Reconstituted detector enzyme conjugate is unstable and should be used immediately. If you wish to run less than a full plate, it should be stored as frozen aliquots at -80°C. Aliquots must be thawed **immediately** before use. After one thaw, any unused detector enzyme conjugate must be discarded. TMB solution should be protected from light.

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 Dextran Sulfate 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 K-4100 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 K-4100 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of high molecular weight dextran sulfate levels in plasma. 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

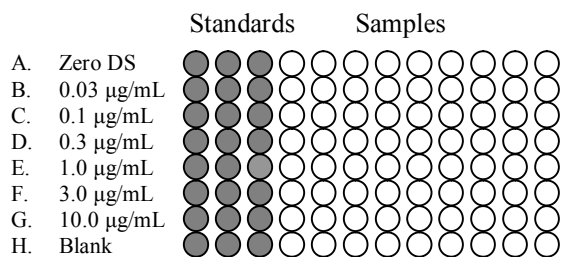
Dextran sulfate Standards: Prepare standards using fresh normal human plasma (platelet depleted) and your dextran sulfate to obtain standards of 0.03, 0.1, 0.3, 1.0, 3.0 and 10.0 µg/mL. **Standardization should be performed using dextran sulfate that is the same dextran sulfate type contained in your unknowns.**

Working Detector-Enzyme Conjugate: Measure exactly **9.1mL** of conjugate diluent PL and add to a clean tube. Perform a 'clean transfer' of the tube of the Detector-Enzyme Conjugate into the 9.1 mL of conjugate diluent PL. 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 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. Reconstituted Detector-Enzyme Conjugate is unstable in the Conjugate Diluent PL and should be

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used immediately. If you wish to perform less than a full plate, the Reconstituted Detector-Enzyme Conjugate can be stored as frozen aliquots at -80°C and thawed immediately before re-use. After one thaw, any unused detector enzyme conjugate must be discarded.

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 **10 µL** of Standards and samples into corresponding wells. Immediately add **90 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 motorized plate rotator is highly recommended for incubations, if available, as this 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.

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