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

Product No: **K-4800**

Dermatan Sulfate ELISA
for buffer or urine

K-4800: Dermatan Sulfate ELISA for Buffer or 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
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)

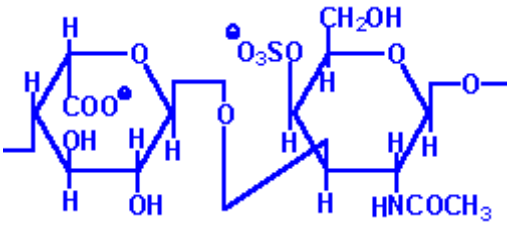
Researcher must provide:

Pipettes (8-channel multipipettor recommended)
Absorbance microplate reader
Dermatan sulfate standards
Tris Buffered Saline (TBS) pH 7.5 (10mM Tris 150mM NaCl)
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



Dermatan sulfate (DS) is in the family glycosaminoglycan. DS is found mostly in the skin but also in blood vessels, tendons, heart valves, and pulmonary connective tissue.

The DS ELISA, product number K-4800, is a quantitative enzyme-linked assay designed for the *in vitro* measurement of DS levels in buffer or urine. This assay measures DS directly using a DS binding protein which has been conjugated to HRP.

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

Crossreactivity

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

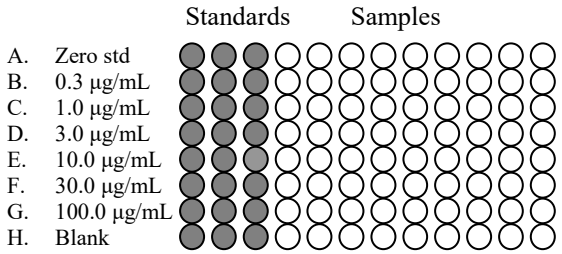
Reagent Preparation

Dermatan Sulfate Standards: Prepare dilutions of your dermatan sulfate in Tris Buffered Saline (TBS) pH 7.5 (10mM Tris, 150mM NaCl) to obtain standards of 0.3, 1.0, 3.0, 10, 30 and 100µg/mL. **Standardization should be performed using DS that is the same DS type contained in your unknowns.**

Working Detector-Enzyme Conjugate: Measure exactly **9.1mL** of conjugate diluent 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. 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 and should be used immediately. If you wish to perform less than a full plate, the reconstituted Detector-Enzyme Conjugate must be stored as aliquots at -80°C. Aliquots must be thawed **immediately** before 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.



Dermatan Sulfate Plate

Assay Procedure

1. Set up the ELISA plate as illustrated above. We suggest the 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 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 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 Dermatan Sulfate)-A_{450}(Blank)] x 100 = % Binding

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