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For Investigational Use Only

Low Molecular Weight Heparin (LMWH) ELISA Kit for Plasma Samples

Product No: K-2000

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

Kit includes:

Heparin-coated 96-well plate
Conjugate Diluent
Detector-Enzyme Conjugate
TMB Solution
Stop Solution, 0.5M H_2SO_4
Wash Concentrate 10X, (dilute 1 part plus 9 parts purified H_2O to make TBS-0.05% TWEEN 20)

Researcher must provide:

Pipettes (8-channel multipipettor recommended)
Absorbance microplate reader
Plate Cover
Normal Plasma

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 4ºC. The TMB solution should be protected from light. Reconstituted detector enzyme conjugate should be used immediately or aliquotted and stored at -80°C.

Background

Heparin is a glycosaminoglycan with alternating uronic acid and aminoglycoside units. It is an anticoagulant used either in its native unfractionated form (UFH) MW ~16 kD or in various partially depolymerized forms (LMWH) of 4-8 kD. The heparin-ELISA product number K-2000 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of low molecular weight heparin levels in plasma. This assay measures heparin directly using a heparin binding protein which has been conjugated to HRP.

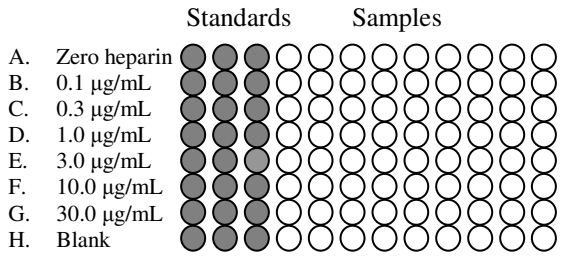
The heparin-ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of heparin present in the sample. Samples to be assayed are first mixed with the Detector-Enzyme Conjugate in wells of the heparin coated plate. Heparin in the sample competes with heparin bound to the plate for binding of the Detector-Enzyme Conjugate. The concentration of heparin in the sample is determined using a standard curve of known amounts of heparin spiked into normal plasma. **For best results, the heparin used for the standard curve should match the type of heparin being assayed.**

Reagent Preparation

Heparin Standards: Prepare standards using fresh normal plasma and your heparin to obtain standards of 0.1, 0.3, 1.0, 3.0, 10.0 and 30.0 µg/mL. **Standardization should be performed using heparin that is the same heparin 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 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 in the vial. 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 and must be used immediately.

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



Heparin ELISA Plate

Assay Procedure

1. Set up the heparin ELISA plate as illustrated above. We suggest the heparin 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. Cover plate and incubate for one hour at room temperature. A rotator is highly recommended, if available, because 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 30-60 minutes waiting for the zero heparin 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:

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

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