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Product No: **K-1900**
Unfractionated Heparin
ELISA for Buffer Samples
Range: 0.03 – 10 µg/ml

K-1900: Unfractionated Heparin ELISA Kit for Buffer/Urine Samples

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

Kit includes:

Heparin 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
UFH standards from USP reference or your heparin
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

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-1900 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of unfractionated heparin levels in low protein content fluids such as buffer or urine. This assay measures heparin directly using a heparin binding protein which has been conjugated to HRP.

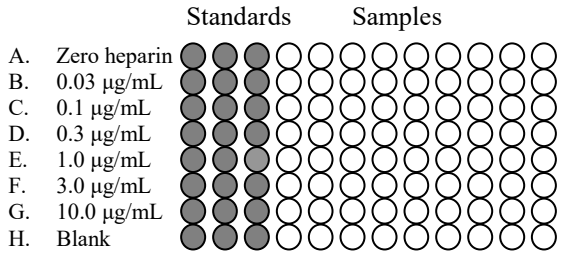
The heparin ELISA is a competitive assay in which the colorimetric signal is inversely proportional to the amount of heparin present in the sample. Samples to be assayed are first mixed with the Detector-Enzyme Conjugate in wells of the heparin coated plate. Heparin in the sample competes with heparin 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.

Reagent Preparation

Heparin Standards: Make dilutions of your heparin in Tris Buffered Saline (TBS) pH 7.5 (10mM Tris, 150mM NaCl) to obtain standards of 0.03, 0.1, 0.3, 1.0, 3.0 and 10.0µg/mL. **Standardization should be performed using heparin that is the same heparin 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 of conjugate diluent. Repeat this step two more times to be sure all the Detector-Enzyme Conjugate has been transferred from the vial to the tube. 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.



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. 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 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 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.