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Product No: **S-1800**
LMW Heparin ELISA Buffer Samples
Liquid Stable Conjugate
Range 0.003 – 1µg/ml

Low Molecular Weight Heparin (LMWH) ELISA Kit for Buffer 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 (stabilized liquid)
TMB Solution
Stop Solution
Wash Concentrate 10X, (dilute 1 part plus 9 parts water)

Researcher must provide:
Pipettes (8 Channel Multipipettor is recommended)
Absorbance microplate reader
LMWH 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. The Detector-Enzyme Conjugate Solution and the TMB solution should be protected from light.

Background

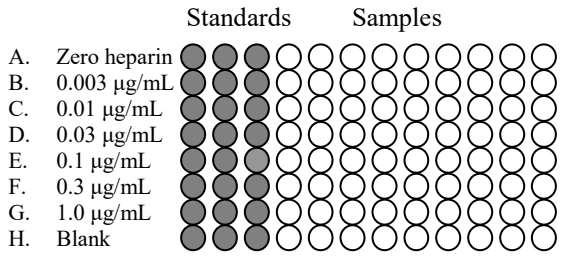
Heparin is a glycosaminoglycan with alternating uronic acid and aminoglycoside units. It is an anticoagulant used either in its native unfractionated form (UFH) MW ~16 kD or in various partially depolymerized forms (LMWH) of 4-8 kD. The heparin-ELISA product number S-1800 is a quantitative enzyme linked assay designed for the *in vitro* measurement of low molecular weight 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

LMW Heparin Standards: Make dilutions of your LMW heparin using Tris Buffered Saline pH 7.5 (10mM Tris, 150mM 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 LMW heparin that is the same LMW heparin type contained in your unknowns.**

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 LMW heparin standard dilution series be run in triplicate for best results. Add **50 µL** of Standards and Samples into corresponding wells. Add **50 µL** of 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, 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 10-60 minutes waiting for the zero LMW 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:

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

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