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Product No: **K-2000**  
Low Molecular Weight Heparin  
ELISA for Plasma Samples  
Range: 0.1 -30 µg/ml

## K-2000: Low Molecular Weight Heparin (LMWH) ELISA Kit for Plasma

**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 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  
LMWH standards from USP reference or your heparin  
Normal 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**

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 or high protein samples. 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.

### **Reagent Preparation**

**Heparin Standards:** Make dilutions of your heparin in plasma (platelet depleted) 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 **9.1 mL** of conjugate diluent PL and add to a clean tube. Perform a 'clean transfer' of the lyophilized 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 containing the 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.

