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Product No: **K-2300**

Unfractionated Heparin (UFH)  
Ultra Range (0.5 U/ml – 150 U/mL)

## Unfractionated Heparin (UFH) Ultra Range ELISA Kit for Buffer 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  
Conjugate Diluent  
TMB Solution  
Stop Solution, 0.5M H<sub>2</sub>SO<sub>4</sub>  
Wash Concentrate 10X, (dilute 1 part plus 9 parts dH<sub>2</sub>O to make TBS-0.05% TWEEN 20)

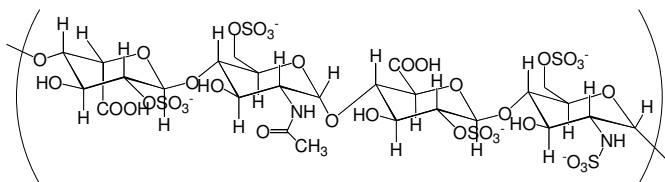
### **Researcher must provide:**

Multichannel Pipetter  
Absorbance microplate reader  
Plate Cover  
UFH Standard from USP reference or your heparin  
Standard Diluent, normal saline (154 mM NaCl)

### **Storage and Stability**

Kit can be stored unopened at 4°C for up to six months. Opened and reconstituted solutions can be used for up to one week when stored at 4°C. 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 (heterogeneous)

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-2300 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. **For best results, the heparin used for the standard curve should match the type of heparin being assayed.**

### **Reagent Preparation**

**Heparin Standards:** Make dilutions of your Heparin Standard using normal saline to obtain standards of 0.3, 1.0, 3.0, 10.0, 30, 100 U/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. 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 can be stored at 4°C for no more than 7 days.

