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Product No: **K-2200**
Unfractionated Heparin
Extended Range ELISA for Buffer Sample
Range: 0.03 – 10 µg/ml

K-2200: Unfractionated Heparin (UFH) Extended 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 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-2200 is a quantitative enzyme-linked assay designed for the *in vitro* measurement of unfractionated heparin levels 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 10.0 µg/mL. **Standardization should be performed using heparin that is the same heparin type contained in your unknowns.**

Working Detector-Enzyme Conjugate: Measure exactly **5.2 mL** of conjugate diluent and add to a clean tube. Perform a 'clean transfer' of the lyophilized Detector-Enzyme Conjugate into the 5.2 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 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.

