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Human Heparin (HEP) ELISA Kit

Part hHEP-ELISA

Assay range

The detection range of this kit is 62.5U/L-2000U/L.

Sensitivity

The sensitivity of this kit is 10U/L.

Introduction

This Quantitative Sandwich ELISA kit allows for the determination of herpain concentrations in human serum, plasma, tissue homogenates and other biological fluids.

Principle

This kit measures human heparin levels using a purified antibody to human heparin, that is coated in microtiter plate wells, making a solid-phase antibody support. Then heparin in standards or samples are added to wells. An HRP labeled detection antibody complex is applied, which generates an antibody-antigen-enzyme-antibody complex. After washing, a TMB substrate solution is added which becomes blue colored. The HRP enzyme-catalyzed, reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at 450 nm. The concentration of heparin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials provided with the kit

Materials provided with the kit	48 determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Microelisa stripplate	1	1	2-8°C
Standard	0.5ml•6 bottle	0.5ml•6 bottle	2-8"C
HRP-Conjugate reagent	5ml•1 bottle	10ml•1 bottle	2-8"C
Sample diluent	3ml•1 bottle	6ml•1 bottle	2-8"C
Chromogen Solution A	3ml•1 bottle	6ml•1 bottle	2-8"C
Chromogen Solution B	3ml•1 bottle	6ml•1 bottle	2-8"C
Stop Solution	3ml•1 bottle	6ml•1 bottle	2-8"C
20 XWash solution	15ml•1 bottle	25ml•1 bottle	2-8"C

Note: Concentration of Provided Standards (U/L)
2000, 1000, 500, 250, 125, 62.5

Brief overview of sample preparation: The following is provided as a general guide for sample preparation. The investigator should perform a literature review to isolate and prepare samples for ELISA

1. Serum: Coagulate at room temperature for 10-20 mins. centrifuge 20-min at 2000-3000 r.p.m. remove supernatant, If precipitation appears, centrifuge again.
2. Plasma: Use EDTA or citrate as an anticoagulant, stir gently for 10-20 mins, centrifuge 20-min at 2000-3000 r.p.m. Remove supernatant, If precipitation appears, centrifuge again.
3. Urine: Collect in a sterile container, centrifuge 20-min at 2000-3000 rpm, remove supernatant. If precipitation appears, centrifuge again.
4. Cell culture supernatant to-detect secretory components, use a sterile container to collect, centrifuge 20-min at 2000-3000 r.p.m and collect the supernatant.
5. Cytoplasmic, internal, non membrane embedded components of cells: Dilute cell suspension with PBS (PH7.2-7.4) with a cell concentration of 1million cells/ml. Perform repeated freeze-thaw cycles to break membrane and release intracellular components. Centrifuge 20-min at 2000-3000 r.p.m. Collect the supernatant to assay. If precipitation appears, centrifuge again.
6. Tissue samples - cut slices, weigh and place in PBS (PH7.2-7.4). Freeze rapidly with liquid nitrogen, maintain samples at 2-8"C after melting. Add PBS (PH7.4). Homogenize then centrifguge for 20-min at the speed of 2000-3000 rpm, remove supernatant.).

7. Avoid sodium azide in samples because it interferes with HRP.

Assay Procedures

(Use a separate and mirror 96-well dish to prepare samples. Transfer all wells simultaneously to the ELISA dish. Prepare samples as noted below).

1. Standards: Set standard wells and add 50ul standard at each concentration.

2. Blank Wells and Samples: Set blank wells separately. For blank wells do not add sample and HRP-Conjugate reagent, otherwise follow all steps. Samples: Premix 40 ul sample diluent with 10 ul sample, then add the 5-fold diluted sample into wells. Do not touch the well wall. Mix gently. Include the 5X dilution factor in the final calculation of sample concentration.

3. Add detection antibody – HRP enzyme conjugate: Add 100ul of detection antibody-HRP-Conjugate reagent to each well, except to the blank well.

4. Incubate: After closing the plate with the closure plate membrane, incubate for 60 min at 37"C.

5. Configure 20-fold wash solution diluted 20-fold with distilled water.

6. Washing: Uncover closure plate membrane, discard Liquid, gently tap drain, add 100 ul wash buffer to every well to 1 min, then drain, repeat 4 times at 30 sec each, tap dry.

7. Add 50ul Chromogen Solution A and 50ul Chromogen Solution B to each well, cover with aluminum foil to limit light exposure and incubate for 15 min at 37"C. This reaction is light sensitive, therefore protect from light.

8. Stop the reaction. Add 50ul Stop Solution to each well, Stop the reaction (the blue color should change to yellow color).

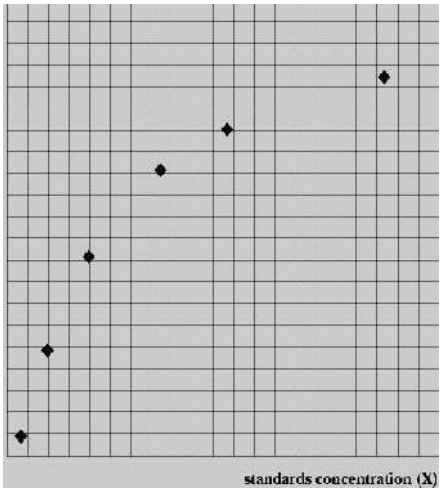
9. Set the blank well as zero, read absorbance at 450nm within 15min of adding the stop solution.

Important notes

1. Equilibrate the reagents and plate for 15-30 minutes at room temperature prior to assaying. Store unused ELISA plate sections in a sealed plastic bag. Do not allow the wells to dry.
2. Resuspend crystallized wash buffer by heating. Equilibrate to RT before applying.
3. Pre-mix samples in mirror 96 well dish and transfer simultaneously to ELISA plate.
4. Remember to tabulate the 5X dilution factor.
5. Do not reuse closure membranes due to cross-contamination.
6. Limit light exposure to the TMB reagents.
7. Do not mix reagents with those from other lots.

Calculate

Set and plot OD on the horizontal axis and concentration on the vertical axis for the standards. Plot the sample ODs and extrapolate the concentration multiplied by 5. Alternatively, calculate a straight line regression equation for the standard curve to estimate the sample concentration.



This chart is for reference only

Assay range

The detection range of this kit is 62.5U/L-2000U/L.

Sensitivity

The sensitivity of this kit is 10U/L.

Storage and validity

Storage : 2-8”C. validity : six months.