HEPARIN CATCHER SURFACE

Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 2.0 to 160.0 U/ml

Reagents

1. Biomat heparin catcher plate code **HC3**
2. UFH (Sigma code H 4784, lot 019K1487 140 USP units/mg)
3. Heparin biotin (Sigma code B 9806, lot 069K1378)
4. Streptavidin peroxidase (BioSpa code SB01-61 at 1mg/ml, lot 00256/1-1)
5. Streptavidin peroxidase diluent
6. TMB Substrate
7. Stop Solution, 0.3 N H_2SO_4
8. Wash Buffer (0.1 M PBS pH 7.2+0.05% Tween$^®$ 20)
9. Standard Diluent (0.1 M PBS pH 7.2)

Reagent preparation

1. <u>Heparin Standards</u>: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 2.0, 10.0, 20.0, 40.0, 80.0, 160.0 U/ml, starting from UFH H 4784. **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. <u>Heparin biotin</u>: Make a 5.0 μg/ml using the Standard Diluent
3. <u>Streptavidin-peroxidase conjugate</u>: Make a 1:25.000 dilution in Streptavidin peroxidase diluent just before the use

Assay Procedure

We suggest to run in duplicate both the heparin standards and samples in order to get the best results.

1. Place the desired number of Biomat heparin catcher coated strips into the holder
2. Dispense 10 μl of Standard Diluent as 0 U/ml standard, heparin standards from 2.0 to 160.0 U/ml and samples into the appropriate wells; immediately after dispense 200 μl of diluted heparin biotin 5.0 μg/ml into each well

Moreover add two wells where to dispense 200 μl of Standard Diluent to be used as NSB (non specific binding)

3. Mix well and incubate for two hours at room temperature
4. Remove liquid from the wells and wash three times with 300 μl of 0.1 M PBS pH 7.2

Blot on absorbance paper or paper towel

5. Dispense 200 μl of streptavidin-peroxidase conjugate to each well and incubate for 1 hour at room temperature
6. Remove streptavidin-peroxidase conjugate from all wells. Wash wells three times with 300 μl of Wash Buffer Blot on absorbance paper or paper towel.
7. Dispense 200 μl of TMB substrate and incubate 15 minutes at room temperature
8. Add 100 μl of Stop solution
9. Read O.D. at 450 nm using an ELISA reader. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of results

Calculate the mean of the duplicate readings for each standard and sample and subtract the mean NSB optical density. Construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a linear x-axis and draw the best fit curve through the points on the graph.

% B/B_0 can be calculated by dividing the corrected O.D. for each standard and sample by the corrected 0 U/ml O.D. standard (B_0) and multiplying by 100.

Calculate the concentration of heparin corresponding to the mean absorbance or the % B/B_0 from the standard curve.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

U/ml	Average mO.D.	Corrected	% B/B$_0$
NSB	167	--	--
0 (B0)	3368	3201	100%
2	3072	2905	90.8%
10	2236	2069	64.6%
20.0	1766	1599	50.0%
40.0	1294	1127	35.2%
80.0	791	624	19.5%
160.0	577	410	12.8%