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HEPARIN CATCHER SURFACE

Background

Heparin is one of the most intensively studied glycosaminoglycans as a result of its anticoagulant properties.
Heparin is used as an anticoagulant either in its native unfractionated form (UFH) MW ~ 16 kD or as partially depolymerized form called low molecular weight (LMW) heparin MW ~ 4-8 kD.

Heparin assay

Biomat developed three Heparin Catcher plates, as special surfaces onto which heparin at different ranges of U/ml can be immobilized.
The proposed assays exploit the different Biomat heparin catcher plates, by quantitative enzyme-linked assays for the *in vitro* measurement of unfractionated heparin in low protein content fluid such as a buffer.
These heparin ELISA tests are competitive assays in which the colorimetric signal is inversely proportional to the amount of heparin present in the sample.

Principle of the assay

Samples to be assayed are at first mixed with a known amount of biotinylated heparin within the wells of Biomat heparin catcher plate.
The Heparin in the sample competes with biotinylated heparin to bind to the binding sites of heparin catcher plate.
After the removal of the unbound reagent and sample, a streptavidin-peroxidase conjugate is added to reveal the reaction.
The concentration in the sample is determined using a standard curve of known amounts of heparin.

Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 0.01 to 2.0 U/ml

Reagents

1. Biomat heparin catcher plate code **HC1**
2. UFH (Sigma code H 4784, lot 019K1487 140 USP units/mg)
3. Heparin biotin (Sigma code B 9806, lot 128K1599)
4. Streptavidin peroxidase (BioSpa code SB01-61 at 1 mg/ml, lot 00255/1-2)
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. Heparin Standards: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 0.01, 0.05, 0.1, 0.5,1.0, 2.0 U/ml, starting from UFH H 4784 **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. Heparin biotin: Make a 1.25 μg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: 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 50 μl of Standard Diluent as 0 U/ml standard, heparin standards from 0.01 to 2.0 U/ml and samples into the appropriate wells; immediately after dispense 50 μl of diluted heparin biotin 1.25 μg/ml into each well. Moreover add two wells where to dispense 100 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 100 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 100 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

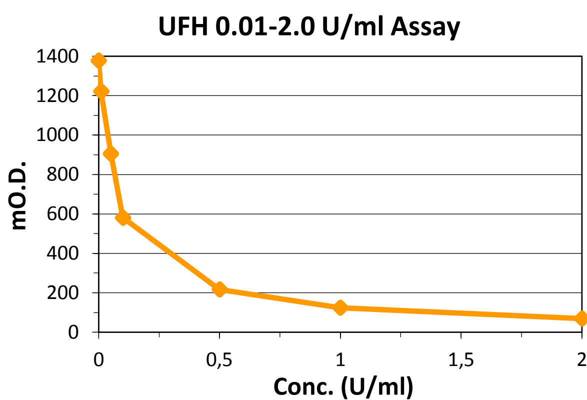
Calculation of results

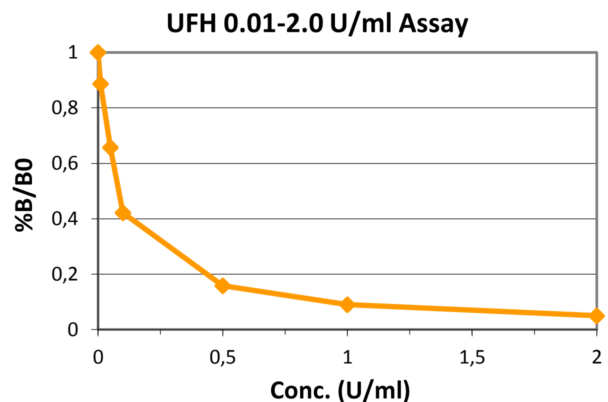
Calculate the mean of the duplicate readings for each standard and sample and subtract the mean NSB optical density. Construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a linear x-axis and draw the best fit curve through the points 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	72	-	-
0 (B_0)	1450	1378	100
0.01	1294	1222	88.7
0.05	978	906	65.7
0.1	653	581	42.2
0.5	289	217	15.8
1.0	196	124	9.0
2.0	141	69	5.0





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Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 0.5 to 40.0 U/ml

Reagents

1. Biomat heparin catcher plate code **HC2**
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. Heparin Standards: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 0.5, 2.5, 5.0, 10.0, 20.0, 40.0 U/ml, starting from UFH H 4784. **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. Heparin biotin: Make a 2.77 μg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: 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 0.5 to 40.0 U/ml and samples into the appropriate wells; immediately after dispense 90 μl of diluted heparin biotin 2.77 μg/ml into each well Moreover add two wells where to dispense 100 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 100 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 100 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.

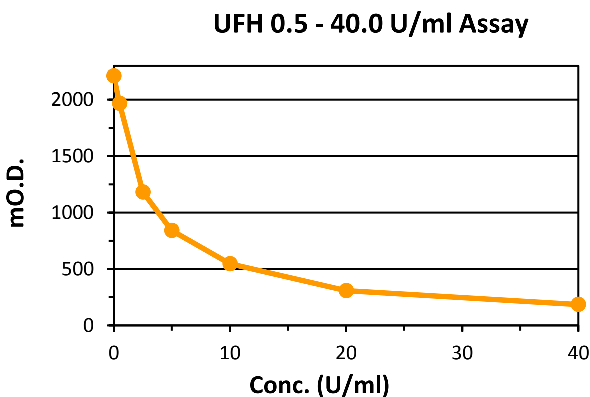
% B/B_0 can be calculated by dividing the corrected O.D. for each standard and sample by the corrected 0 U/ml O.D. standard (B_0) and multiplying by 100.

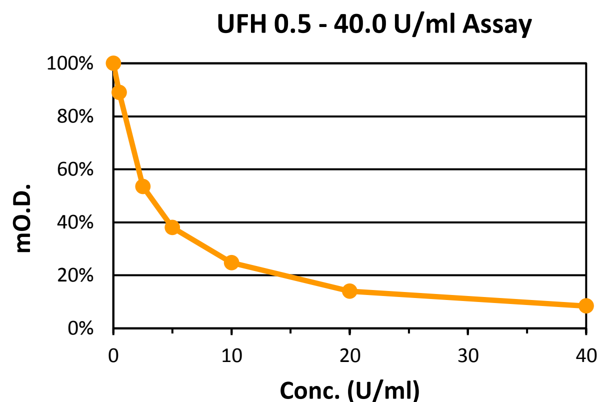
Calculate the concentration of heparin corresponding to the mean absorbance or the % B/B_0 from the standard curve.

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	116	-	-
0 (B_0)	2326	2210	100%
0.5	2082	1966	89.0%
2.5	1298	1182	53.5%
5.0	956	840	38.0%
10.0	661	545	24.7%
20.0	425	309	14.0%
40.0	301	185	8.4%





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

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. Heparin Standards: 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. Heparin biotin: Make a 5.0 μg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: 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 (B_0)	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%