Echelon Biosciences Inc.

Heparin ELISA Kit

K-5300 (96 tests)

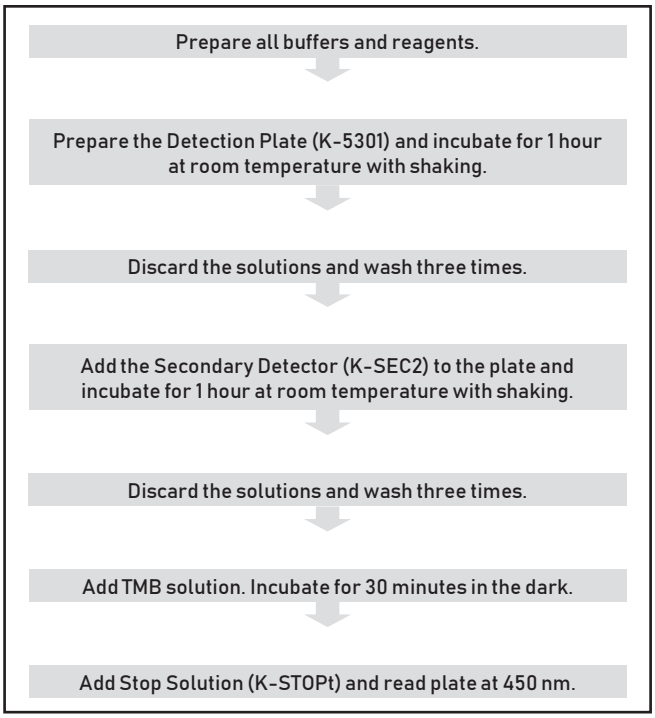
Support: echelon@echelon-inc.com

Description: Quantitative immunoassay designed for in vitro measurement of heparin in purified/synthetic samples

Materials Provided

Catalog #	Description	Quantity
K-5301	Heparin Detection Plate	1 plate
K-5302	Heparin Detector	1 bottle
K-5303	Heparin Standard (Porcine)	1 vial
K-5304	Detector Diluent (7 mL)	1 bottle
K-SEC2	Secondary Detector (300 µL)	1 vial
K-PBST3	10X PBS-T Solution (30 mL)	1 bottle
K-PTAB	PBS Tablets	2 tablets
K-TMB1	TMB Solution (12 mL)	1 bottle
K-STOPt	1N H_2SO_4 Stop Solution (10 mL)	1 bottle
---	Microtiter plate seal	2 seals

Quick Protocol



Additional Materials Provided by User:

- Microtiter plate reader capable of reading absorbance at 450 nm.
- Pipettes (20 µL, 200 µL, and 1,000 µL)
- 0.5 mL or 1.5 mL centrifuge tubes
- Bottle with volume >400 mL
- Reagent grade water

Storage: Upon receipt store kit at 4 °C.

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Background

Heparin is a highly sulfated glycosaminoglycan ranging in size from 6 to 20 kDa. Heparin is presented in mast cells, liver and lungs. Although its function is unknown, it is widely used as an anticoagulant to prevent blood clotting *in vivo* and *in vitro*. For example, heparin is used as preventative treatment for deep vein thrombosis and pulmonary embolism. Oversulfated chondroitin sulfate contamination in raw heparin stocks used for injectable heparin manufacturing was discovered in 2008 and resulted in a major recall of related medical products. According to the FDA, it is believed at least 11 deaths were associated with the contaminated heparin-containing devices worldwide. Therefore, ensuring purity of raw heparin is critical in medical heparin manufacturing.

Assay Design

Echelon's Heparin ELISA is a quantitative immunoassay designed for *in vitro* measurement of heparin in purified/synthetic samples. The concentration of heparin in the sample is determined using a standard curve of known amounts of heparin in a competitive ELISA format. The heparin in the sample competes with heparin bound to the plate for binding to the Heparin Detector. Therefore, the assay signal is inversely proportional to the amount of heparin present in the sample. Echelon's Heparin ELISA provides a robust and simple method for researchers to measure heparin or identify contaminated heparin stock.

Assay Kit Notes

1. Echelon's Heparin ELISA is designed for one-time use only. Collect enough samples to ensure the kit is fully utilized.
2. If sample dilutions are necessary, use the same buffer found in the original sample as diluent for the most accurate results. If the same buffer is not available, PBS (provided in kit, K-PTAB) can be used as diluent but results may vary. Always use the same sample diluent and the same dilution factor in order to achieve the best assay coefficient of variation between experiments.
3. Assay interference by chondroitin sulfate, dermatan sulfate (chondroitin sulfate B), dextran sulfate and hyaluronic acid were evaluated. No cross-reactivity was observed when testing hyaluronic acid. However, the Heparin ELISA does cross-react with dextran sulfate. The Heparin ELISA also cross-reacts significantly with chondroitin sulfate and dermatan sulfate. The competitive IC50 curves are at least 50X less sensitive for chondroitin sulfate and dermatan sulfate compared to heparin. Until all factors have been tested, the possibility of interference cannot be excluded.
4. Samples must be added into the Heparin Detection Plate (K-5301) before adding the Heparin Detector (K-5302). Expect a reduction in assay sensitivity if the Heparin Detector is added before the samples. Always run the Heparin ELISA using the same protocol to achieve the best assay coefficient of variation between experiments.
5. Shaking is necessary during the incubation step in order to achieve the intended assay sensitivity. If a plate shaker is not available, tap plate to mix after adding samples and the Heparin Detector before timing the incubation but expect a reduction in assay signal and sensitivity. Always run the Heparin ELISA using the same protocol to achieve the best assay coefficient of variation between experiments.
6. The data is best analyzed by fitting the Heparin Standards using the "Sigmoidal Doses Response" curve fit on a semi-log plot. Alternative curve fit such as the linear regression with log-log plot can also be used. The same method of analysis should be used between experiments for accurate comparison.
7. If the lyophilized Heparin Standard (K-5303) is brought up with a different volume it will produce a curve of altered sensitivity. Once in solution, the standard can be diluted differently than is suggested. See step 5 in the assay protocol for information on how to properly solubilize the Heparin Standard.

Assay Protocol

Please read this entire section and the assay notes section before beginning the assay.

1. Reconstitute the Heparin Detector (K-5302) with 6 mL Detector Diluent (K-5304). Invert bottle up and down to mix. Keep at room temperature until use. This preparation is stable and must be at room temperature before addition to the plate (step 7). This preparation can be stored at 4°C for at least 3 months.
2. Warm kit reagents to room temperature for 1 hour before step 5. Place Heparin Detector (K-5302) and Secondary Detector (K-SEC2) on ice until use. Temperature is very important in this assay. Running the assay with low temperatures will produce a less sensitive curve.
3. Prepare PBS by dissolving each PBS tablet (K-PTAB) with 200 mL reagent grade water. Keep at room temperature.
4. Prepare 1X PBS-T solution by adding 30 mL of the 10X PBS-T solution (K-PBST3) to 270 mL of reagent grade water. Keep at room temperature.
5. Reconstitute the Heparin Standard (K-5303) with 50 µL water for 10 mg/mL. Vortex to ensure Heparin Standard is fully reconstituted. Then serial dilute 1:10 according to Table 1. Keep standard at room temperature. Proceed immediately to next step.

Table 1. Heparin Standards

Row	Heparin Concentration	Heparin Standard or Previous Dilution Needed	1X PBS Needed
B	100,000 ng/mL	10 µL of 10,000,000 ng/mL (10 mg/mL)	990 µL
C	10,000 ng/mL	30 µL of 100,000 ng/mL	270 µL
D	1,000 ng/mL	30 µL of 10,000 ng/mL	270 µL
E	100 ng/mL	30 µL of 1,000 ng/mL	270 µL
F	10 ng/mL	30 µL of 100 ng/mL	270 µL
G	1 ng/mL	30 µL of 10 ng/mL	270 µL
H	0.1 ng/mL	30 µL of 1 ng/mL	270 µL

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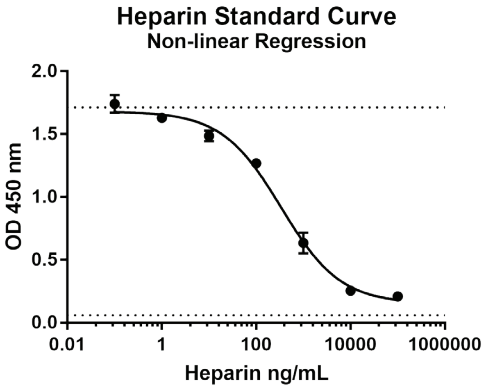
6. Remove the Heparin Detection Plate (K-5301) from plastic bag. Add 50 µL/well of the prepared standards (step 5) or samples to the Heparin Detection Plate (K-5301) according to Table 2. For No Heparin and Blank wells add 50 µL/well of PBS (step 3). See assay note #2 for sample dilution information.
7. Add 50 µL/well of the reconstituted Heparin Detector (step 1) into the Heparin Detection Plate (K-5301) to all wells except Blank control. To blank control add 50 µL/well of Detector Diluent (K-5304). Cover plate with plate seal. Incubate at room temperature for 1 hour WITH SHAKING. See assay note #4 & #5 for Heparin Detector addition and plate shaking information.
8. Discard solution from plate, wash plate three times with 200 µL of PBS per well. Leave last wash in plate.
9. Prepare the Secondary Detector by adding 130 µL of the Secondary Detector stock (K-SEC2) to 12 mL of 1X PBS-T solution. Discard last wash. Add 100 µL/ well of the diluted Secondary Detector. Cover plate with plate seal. Incubate at room temperature for 1 hour WITH SHAKING.
10. Discard solution from plate, wash plate three times with 200 µL of PBS per well. Remove last wash from plate.
11. Add 100 µL TMB solution per well (K-TMB1). Let blue color develop for approximately 30 minutes. PROTECT FROM LIGHT.
12. Add 50 µL 1 N H2SO4 solution (K-STOPt) to each well to stop the reaction. Tap plate to mix.
13. Read absorbance at 450 nm.
14. Generate a best fit curve for the heparin standards in order to interpolate relative sample values. See Figure 1 and assay note #6 for more information.

Figure 1



Heparin standard curve was generated using the "log(agonist) vs. response -- Variable slope (four parameters)" curve fit in GraphPad Software by plotting the x-axis in log scale. The top and bottom were constrained using the 0 ng/mL and blank values from the heparin standard curve.

References

1. Cai S, Dufner-Beattie JL, Prestwich GD. (2004) A selective protein sensor for heparin detection. Anal Biochem. 326(1):33-41.

Related Products

Products	Catalog Number
Other ECM Assays	
Hyaluronidase Activity	K-6000
HA Competitive ELISA	K-1200
HA Sandwich ELISA	K-4800
Collagen IV	K-1700s
Select-HA	
Select-HA 1000k	HYA-1000KEF-1
Select-HA 500k	HYA-500KEF-1
Select-HA HiLadder	HYA-HILAD-20
Select-HA LoLadder	HYA-LOLAD-20

Table 2. Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Blank*	*Blank*	*No Heparin*	*No Heparin*	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
B	100,000 ng/mL	100,000 ng/mL	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
C	10,000 ng/mL	10,000 ng/mL	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
D	1,000 ng/mL	1,000 ng/mL	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
E	100 ng/mL	100 ng/mL	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
F	10 ng/mL	10 ng/mL	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
G	1 ng/mL	1 ng/mL	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
H	0.1 ng/mL	0.1 ng/mL	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39

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