Echelon Biosciences Inc.

Hyaluronidase Activity ELISA

K-6000 (Kit = 1 X 96 wells, Pack = 5 X 96 wells)

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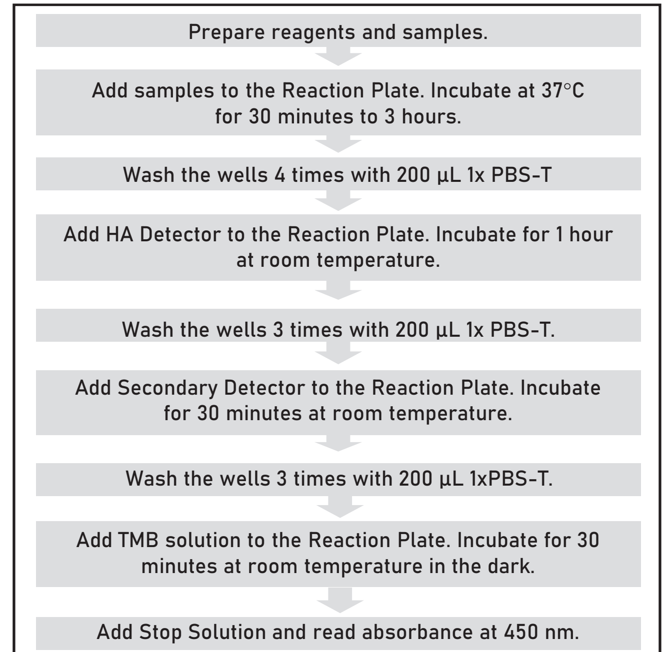
Description: 96-well ELISA Assay for Detection and Quantification of Hyaluronidase Activity

Materials Provided

Catalog #	Description	Kit	Pack
K-6001	Reaction Plate	1 Plate	5 Plates
K-6002	5X Reaction Buffer, pH 4.2	1 Bottle	5 Bottles
K-6003	HA Detector	1 Bottle	5 Bottles
K-6004	Secondary Detector	1 Bottle	5 Bottles
K-6005	Hyaluronidase Control	1 Vial	5 Vials
K-PBST3	10X PBS-T Buffer	1 Bottle	5 Bottles
K-GS01	Protein Stabilizer	1 Vial	5 Vials
K-TMB1	TMB Solution	1 Bottle	5 Bottles
K-STOPt	1N H_2SO_4 Stop Solution	1 Bottle	5 Bottles
---	Microtiter plate seal	3 Seals	15 Seals

Quick Protocol



Additional Materials Provided by User

- Microtiter plate reader capable of reading absorbance at 450 nm
- Incubator set at 37°C
- Plate shaker or similar
- Pipettes (20 µL, 200 µL, and 1,000 µL)

Storage

- Kit - store at -20°C
- Pack - store Part 1 at -20°C and Part 2 at -20 to 4°C

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Background

Hyaluronidases are a group of enzymes that degrade Hyaluronan (HA), a linear polysaccharide comprised of a repeating disaccharide of N-acetylglucosamine and D-glucuronic acid. HA is involved in many biological processes including structural support, cell migration and tissue turnover. Circulating HA levels have also been shown to correlate in several disorders such as liver disease and certain types of cancer. Hyaluronidases have become an important area of study due to their regulatory function in HA metabolism and their role in physiological processes such as fertilization and wound healing. Research has also shown that hyaluronidases are involved in several pathological processes such as bacterial pathogenesis, the spreading of toxins/venoms and cancer progression, making hyaluronidases a potential pharmacological target.

Assay Design

Echelon's Hyaluronidase Activity ELISA is a quantitative immunoassay designed for in vitro measurement of hyaluronidase activity in biological samples. Hyaluronidase reactions are performed in a 96-well microtiter plate pre-coated with HA substrate. The activity of the hyaluronidase is determined by comparing HA substrate levels post reaction to a standard curve of pre-coated HA substrate amounts. Echelon's Hyaluronidase Activity ELISA has been validated with human sera, plasma and urine samples in addition to purified hyaluronidase from bovine testes. Echelon's Hyaluronidase Activity ELISA provides a robust and simple method for researchers to measure hyaluronidase activity in biological samples.

Assay Notes

1. The 5X Reaction Buffer (K-6002) must be used for the enzyme reactions. It is optimized for hyaluronidases requiring acidic conditions. The pH of the reaction buffer should be adjusted, with NAOH, if samples require neutral conditions.
2. The Reaction Plate (K-6001) has been coated with HA with a MW of 1.5-1.8 10^6 Daltons. Do not let the Reaction Plate dry out once the assay has started. Always prepare the next solution needed prior to discarding the previous solution.
3. All reagents should be used immediately once they are diluted to working concentrations and cannot be saved for future use.
4. Running the Hyaluronidase Control is optional but recommended. A minimum of 1 duplicate data point should be run. The Hyaluronidase Control (K-6005) is sourced from bovine testes. It is Hyaluronidase type I-S. The Hyaluronidase control can be run as a 7 point titration as follows. 1- Prepare a 3000 mU/mL stock of Hyaluronidase by adding 335 µL 1X Reaction Buffer to the vial of Hyaluronidase Control (K-6005). Mix gently to fully dissolve the enzyme. 2- Serial dilute the Hyaluronidase 3-fold, 6 times in microcentrifuge tubes. Run the reaction for 2 hours at 37°C.

Sample Preparation

1. Samples should be free of any debris that may interfere with the assay.
2. A sample dilution of 20-fold is the minimum dilution recommended. Hyaluronidase activity can vary depending on the sample, so testing multiple dilutions may be beneficial.
3. When testing plasma, avoid using plasma prepared with heparin.
4. Sample buffers/diluents should be run as controls in the assay.

Assay Protocol

Please read this entire section, Assay Notes, and Sample Preparation sections before beginning.

1. Bring kit reagents to room temperature before use except the HA Detector (K-6003), Secondary Detector (K-6004) and Hyaluronidase Control (K-6005). Keep these reagents on ice until use.
2. Prepare 1X PBS-T buffer by diluting 30 mL of the 10X PBS-T Buffer (K-PBST3) with 270 mL dH2O.
3. Prepare 1X Reaction Buffer by diluting 4 mL of the 5X Reaction Buffer (K-6002) with 16 mL dH2O.
4. Prepare the Hyaluronidase Control (K-6005) by adding 1 mL of the 1X Reaction Buffer to the vial for a 1 U/mL concentration. Mix gently to fully resuspend the enzyme. The enzyme should be diluted just prior to use. See assay notes 4
5. Prepare samples by diluting 20-fold with 1X Reaction Buffer. See "Sample Preparation" section. It is recommended that samples are run in duplicate. See suggested plate layout. (Table 1). Samples should be diluted just prior to use.
6. Add 100 µL/well of the diluted samples and Hyaluronidase Control to the Reaction Plate (K-6001) according to the plate layout below. Add 100 µL/well of the 1X Reaction Buffer to HA standard wells and "No Hyaluronidase" control in duplicate.
   Columns 1-2 of the Reaction Plate are pre-coated with an HA standard curve. The remaining columns are coated with 5 µg HA substrate/well and are for samples and controls. No samples or controls should be added to the HA standard columns. See assay notes 2.
7. Cover the Reaction Plate with a plate seal. Incubate the reaction at 37°C. Incubation times may vary depending on the Hyaluronidase activity within samples. Suggested incubation time –30 minutes to 3 hours.
8. After incubation, prepare the HA Detector working solution by adding 12 mL 1X PBS-T buffer and 120 µL Protein Stabilizer (K-GS01) to the bottle of HA Detector (K-6003). Mix gently to fully resuspend the protein. Place prepared HA Detector at room temperature and proceed immediately to the next step. Do not leave prepared HA Detector at room temperature for more than 10 minutes.
9. Discard solution from plate and wash 4X with 200 µL/well 1X PBS-T buffer. Add 100 µL of the diluted HA Detector (step 8) to all wells of the Reaction Plate. Cover plate with a plate seal and incubate for 1 hour at room temperature on a plate shaker.
10. After the incubation, prepare the Secondary Detector working solution by adding 12 mL of PBS-T to the bottle of Secondary Detector (K-6004). Mix gently to fully resuspend the Secondary Detector and set at room temperature until use. Do not leave prepared Secondary Detector at room temperature for more than 10 minutes.
11. Discard solution from plate and wash 3X with 200 µL/well 1X PBS-T buffer. Add 100 µL of the diluted Secondary Detector (step 10) to all wells of the Reaction Plate. Cover plate with a plate seal and incubate for 30 minutes at room temperature on a plate shaker.
12. After the incubation, wash plate three times with 200 µL 1X PBS-T buffer per well.
13. Add 100 µL of the TMB solution (K-TMB1) to each well of the Reaction Plate. Let blue color develop for 30 minutes in the dark.
14. Add 50 µL of the 1 N H2SO4 Stop Solution (K-STOPt) to each well to stop the reaction. Tap plate to mix.
15. Read absorbance at 450 nm.

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Table 1, Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µg	5 µg	HAase Control	HAase Control	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31
B	2.5 µg	2.5 µg	No HAase	No HAase	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
C	1.25 µg	1.25 µg	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
D	0.625 µg	0.625 µg	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
E	0.313 µg	0.313 µg	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
F	0.156 µg	0.156 µg	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
G	0.078 µg	0.078 µg	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
H	No HA	No HA	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38

Data Analysis

Generate a best fit curve for the HA substrate standards and interpolate the remaining HA substrate in the reaction wells. Hyaluronidase activity can then be determined based on amount of HA substrate removed during the reaction time period.

Graph the standard curve (column 1 and 2 of the plate) as OD vs Log µg HA remaining. Then fit a nonlinear 4-parameter curve. The sample OD values are then interpolated against the standard curve. Once the amount of HA remaining for each samples is determined, it can be plugged into the equation below.

% HA removed = (1- (ug HA remaining in sample/5ug)) X 100

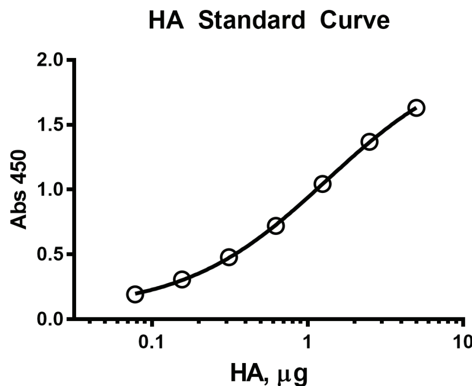

Figure 1.
HA standard curve was generated using non-linear regression analysis with GraphPad Software. A log[agonist] vs. response-variable slope (four parameter) analysis was utilized.

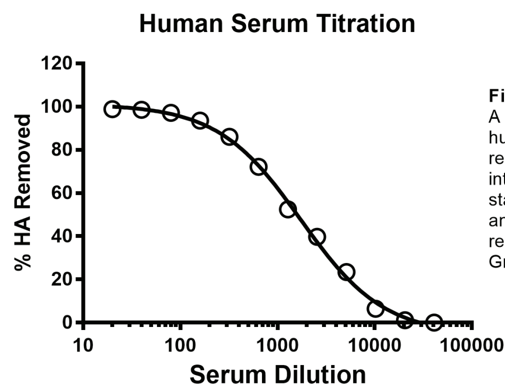

Figure 2.
A sample titration using human serum with a 2 hour reaction time. Data interpolated against an HA standard curve and analyzed using non-linear regression analysis with GraphPad Software.

References

1. Chang S-H, Yeh Y-H, Lee J-L, Hsu Y-J, Kuo C-T, Chen W-J. Transforming growth factor-β-mediated CD44/STAT3 signaling contributes to the development of atrial fibrosis and fibrillation. Basic Research in Cardiology. 2017;112(5):58. doi: 10.1007/s00395-017-0647-9.
2. Margraf A, Herter JM, Kühne K, Stadtmann A, Ermert T, Wenk M, et al. 6% Hydroxyethyl starch (HES 130/0.4) diminishes glycocalyx degradation and decreases vascular permeability during systemic and pulmonary inflammation in mice. Critical Care. 2018;22(1):111.

Related Products

Catalog #	Products
HA Detection Assays	
K-5800	Hyaluronic Acid (HA) Alphascreen
K-4800	Hyaluronic Acid (HA) Sandwich ELISA
K-1200	Hyaluronan (HA) ELISA
HA Binding Proteins	
G-HA01	Versican G1 Domain
G-HA02	Biotinylated Versican G1 Domain
G-HA03	His-tagged Versican G1 Domain
HA Molecular weight Ladders	
HYA-LOLAD	Select HA LoLadder
HYA-HILAD	Select HA HiLadder
HYA-MGLAD	Select HA Mega Ladder

Please visit our website at www.echelon-inc.com for more enzyme and lipid products.

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