Echelon Biosciences Inc.

Hyaluronic Acid (HA) Sandwich ELISA

K-4800 (96 tests)

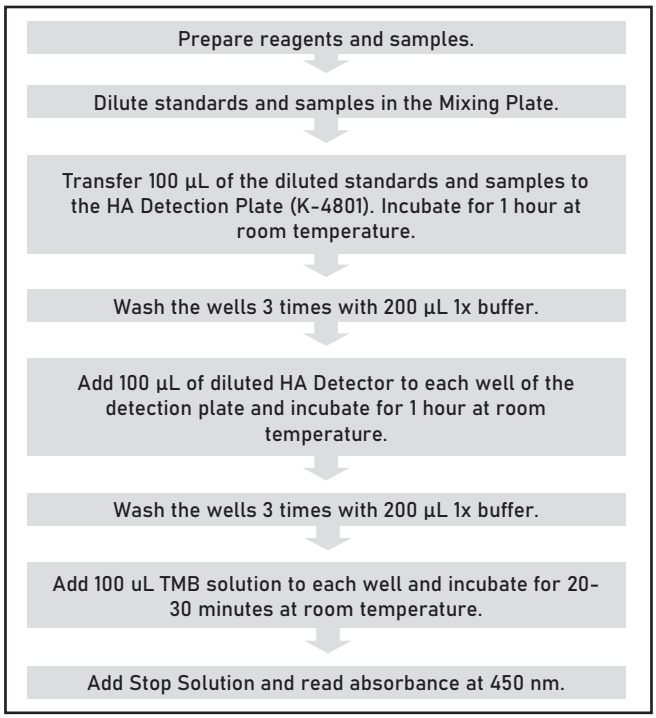
Support: echelon@echelon-inc.com

Description: The HA Sandwich ELISA is for the quantification of HA in low volume (25 µL) samples with HA > 130 kDa.

Materials Provided

Catalog #	Description	Amount
K-4801	Hyaluronic Acid Detection Plate	1 plate
K-4802	Hyaluronic Acid Detector	1 bottle
K-4803	HA Standard (1.6 ug)	1 vial
K-TBST3	10X Assay Buffer (10 mL)	1 bottle
K-TBS1	10X Buffer	1 bottle
K-TMB1	TMB Solution (12 mL)	1 bottle
K-STOPt	1N H_2SO_4 Stop Solution (10 mL)	1 bottle
---	96-Well Mixing Plate (yellow)	1 plate
---	Microtiter plate seal	2 seals

Quick Protocol



Additional Materials Provided by User

- Microtiter plate reader capable of reading absorbance at 450 nm
- Plate shaker or similar
- Pipettes (20 µL, 200 µL, and 1,000 µL)
- Reagent grade water

Storage

Upon receipt store kit at -20°C. Allow the reagents to warm to room temperature before opening vials.

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

Background

Hyaluronan (HA) is a linear polysaccharide comprised of a repeating disaccharide of N-acetylglucosamine and D-glucuronic acid. The major function of HA is to provide structural support of tissue as part of the extracellular matrix (ECM). Thus, HA is widely presented in connective tissue in higher animals. The size of HA varies from 100 kD to 10,000 kD and is responsible for different functions. In humans, free HA enters circulation through the lymph node, where 80% is degraded and recycled by the liver and the remaining 20% is metabolized in the kidneys and excreted through urine. Multiple studies have shown that high serum HA levels correlates with liver

Assay Design

Echelon Bioscience's (EBI) HA Sandwich ELISA is a quantitative immunoassay designed for in vitro measurement of HA levels in cell culture supernatant, or human/animal biological fluids. The concentration of HA in the sample is determined using a standard curve of known amounts of HA. The HA Sandwich ELISA was validated with human sera samples. Only a small amount of sample (25 µL) is needed for running duplicate measurements. The HA Sandwich ELISA provides a robust and simple method for researchers to measure HA in biological samples.

Assay Notes

1. This assay is sensitive to the size of HA contained within the sample. A minimum HA size of ~130,000 daltons is required for detection in the assay.
2. Over development during TMB incubation might result in high background and inconsistent results.
3. Once diluted the HA Detector (K-4802) should be used immediately and cannot be saved for future use. Additional aliquots can be purchased if necessary.

Assay Protocol

Please read this entire section and Assay Notes before beginning the assay.

1. The assay kit should be brought to room temperature before use except for the HA Detector (K-4802) which should be kept at -20°C until use.
2. Prepare 1X Buffer by diluting 1 bottle of the 10X Buffer (K-TBS1) with 180 mL reagent grade water. 1X Buffer is used as wash buffer.
3. Prepare 1X Assay Buffer by diluting 4 mL of the 10X Assay Buffer (K-TBST3) with 36 mL reagent grade water. 1X Assay Buffer must be prepared fresh before use.
4. Prepare the HA Standard (K-4803) by adding 500 µL of reagent grade water to the vial for 3200 ng/mL. Vortex for 30 seconds to completely dissolve the HA. Briefly centrifuge to collect solution. The 3200 ng/mL HA standard can be stored at -20C for up to 3 months. Longer storage of the hydrated HA standard will show lower signal in the assay.
5. Serially dilute the reconstituted HA Standard (K-4803) 4-fold in water, 4 times: 100 uL previous dilution + 300 µL water.
6. Dilute the standards or samples with 1X Assay Buffer in mixing plate (10-fold dilution). Adjust samples, standards and 1X Assay Buffer volume if replicates other than duplicates are preferred. Suggested mixing plate layout is shown below.
   a. Add 225 µL of 1X Assay Buffer into each well of column 1 through 6 in the mixing plate
   b. Add 25 µL of each HA standard (K-4803) or samples into each well of the mixing plate
   c. Use plate shaker or tap plate briefly to mix.
7. Remove the HA Detection Plate (K-4801) from plastic bag.

Table 1, Suggested Mixing Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard S1	Sample #3	Sample #11	Sample #19	Sample #27	Sample #35	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
B	Standard S2	Sample #4	Sample #12	Sample #20	Sample #28	Sample #36	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
C	Standard S3	Sample #5	Sample #13	Sample #21	Sample #29	Sample #37	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
D	Standard S4	Sample #6	Sample #14	Sample #22	Sample #30	Sample #38	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
E	Standard S5	Sample #7	Sample #15	Sample #23	Sample #31	Sample #39	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
F	Assay Buffer	Sample #8	Sample #16	Sample #24	Sample #32	Sample #40	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
G	Sample #1	Sample #9	Sample #17	Sample #25	Sample #33	Sample #41	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-
H	Sample #2	Sample #10	Sample #18	Sample #26	Sample #34	Sample #42	-empty-	-empty-	-empty-	-empty-	-empty-	-empty-

Table 2, Suggested HA Detection Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard S1	Standard S1	Sample #3	Sample #3	Sample #11	Sample #11	Sample #19	Sample #19	Sample #27	Sample #27	Sample #35	Sample #35
B	Standard S2	Standard S2	Sample #4	Sample #4	Sample #12	Sample #12	Sample #20	Sample #20	Sample #28	Sample #28	Sample #36	Sample #36
C	Standard S3	Standard S3	Sample #5	Sample #5	Sample #13	Sample #13	Sample #21	Sample #21	Sample #29	Sample #29	Sample #37	Sample #37
D	Standard S4	Standard S4	Sample #6	Sample #6	Sample #14	Sample #14	Sample #22	Sample #22	Sample #30	Sample #30	Sample #38	Sample #38
E	Standard S5	Standard S5	Sample #7	Sample #7	Sample #15	Sample #15	Sample #23	Sample #23	Sample #31	Sample #31	Sample #39	Sample #39
F	Assay Buffer	Assay Buffer	Sample #8	Sample #8	Sample #16	Sample #16	Sample #24	Sample #24	Sample #32	Sample #32	Sample #40	Sample #40
G	Sample #1	Sample #1	Sample #9	Sample #9	Sample #17	Sample #17	Sample #25	Sample #25	Sample #33	Sample #33	Sample #41	Sample #41
H	Sample #2	Sample #2	Sample #10	Sample #10	Sample #18	Sample #18	Sample #26	Sample #26	Sample #34	Sample #34	Sample #42	Sample #42

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

8. Transfer 100 µL of the diluted samples and standards from the mixing plate to the HA Detection Plate (K-4801). For more consistent results, fully mix the standards/samples with pipette before transferring. Suggested HA Detection Plate layout is shown in Table 1. Cover plate with new plate seal. Incubate at room temperature for 1 hour with gentle agitation on a plate shaker.
9. After incubation, add 12 mL of the 1X Assay Buffer into the HA Detector bottle (K-4802). Gently mix solution and place at room temperature. Wash plate three times with 200 uL 1X Buffer. Add 100 uL of the diluted HA Detector (K-4802) to each well of the HA Detection Plate (K-4801). Cover plate with new plate seal. Incubate at room temperature for 1 hour with gentle agitation on a plate shaker. Once diluted the HA Detector must be used immediately and cannot be stored for future use.
10. After incubation, wash plate three times with 200 µL 1X Buffer per well.
11. Add 100 µL TMB solution per well (K-TMB1). Let blue color develop for approximately 20 to 30 minutes. Do not over develop.
12. Add 50 µL of 1N H_2SO_4 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 standards in order to interpolate relative sample values (see figure 1).

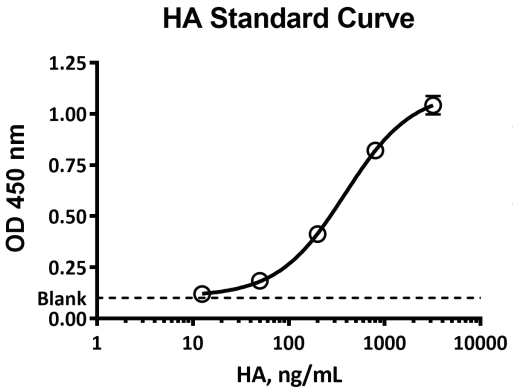


Figure 1. HA standard curve was generated using non-linear regression analysis with GraphPad Software. A log[agonist] vs. response-variable slope (four parameter) analysis was utilized.

References

1. Rivas F, Zahid OK, Reesink HL, Peal BT, Nixon AJ, DeAngelis PL, et al. (2018) Label-free analysis of physiological hyaluronan size distribution with a solid-state nanopore sensor. Nat Commun. 9(1):1037.
2. Vazquez-Portalatin N, Kilmer CE, Panitch A, Liu JC. (2016) Characterization of Collagen Type I and II Blended Hydrogels for Articular Cartilage Tissue Engineering. Biomacromolecules.
3. Fanhchaksai K, Okada F, Nagai N, Pothacharoen P, Kongtawelert P, Hatano S, et al. (2016) Host stromal versican is essential for cancer-associated fibroblast function to inhibit cancer growth. International Journal of Cancer. 138(3):630-41.
4. Sun YY, Keller KE. (2015) Hyaluronan cable formation by ocular trabecular meshwork cells. Experimental Eye Research. 139:97-107.
5. Qin J, Kilkus J, Dawson G. (2015) The hyaluronic acid inhibitor 4-methylumbelliforone is an NSMase2 activator-A novel antitumor mechanism of MU. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 1861(2):78-90.
6. Bruegger D, Brettner F, Rossberg I, Nussbaum C, Kowalski C, Januszewska K, et al. (2015) Acute Degradation of the Endothelial Glycocalyx in Infants Undergoing Cardiac Surgical Procedures. The Annals of Thoracic Surgery. 99(3):926–31.
7. Lennon FE, Mirzapoiazova T, Mambetsariev N, Mambetsariev B, Salgia R, Singleton PA. (2014) Transactivation of the receptor-tyrosine kinase ephrin receptor A2 is required for the low molecular weight hyaluronan-mediated angiogenesis that is implicated in tumor progression. J Biol Chem. 289(35):24043-58.

Related Products

Catalog #	Products
Assays and Services	
T-1200	Hyaluronic Acid (HA) Screening Service
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
Fluorescently Conjugated HA	
H-025F, H-025R	HA30 BODIPY, Texas Red
H-250F, H-250R	HA300 BODIPY, Texas Red
H-700F, H-700R	HA850 BODIPY, Texas Red

Catalog #	Products
Hyalose – Select HA	
HYA-1000KEF-1	Select HA 1000k
HYA-500KEF-1	Select HA 500k
HYA-50KEF-1	Select HA 50k
HYA-1000KEF-1	Biotinylated Select HA 1000k
HYA-500KEF-1	Biotinylated Select HA 500k
HYA-50KEF-1	Biotinylated Select HA 50k
Hyalose – HA Ladders	
HYA-HILAD-20	Select HA HiLadder
HYA-LOLAD-20	Select HA LoLadder
HYA-MGLAD-20	Select HA Mega Ladder

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

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com