Echelon Biosciences Inc.

Hyaluronic Acid (HA) AlphaScreen™ Assay

K-5800 (500 tests)

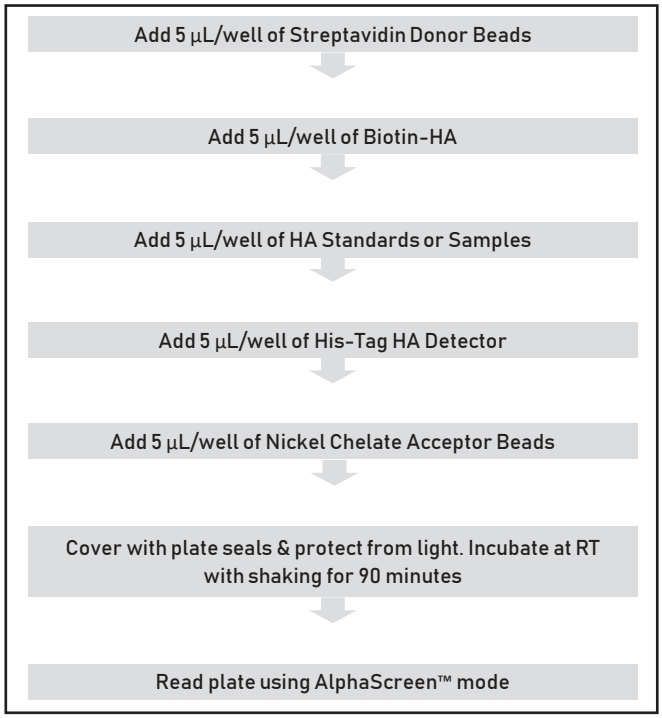
Support: echelon@echelon-inc.com

Description: 500-point AlphaScreen™ assay designed to detect HA purified from biological samples

Materials Provided

Catalog #	Description	Amount
K-5801	His-tag HA Detector (lyophilized)	1 vial
K-5802	Biotin HA (lyophilized)	1 vial
K-1202	HA Standard (3200 ng/mL, 1 mL)	1 vial
K-PBSTB	5X Diluent (10 mL)	1 bottle
---	White Opaque 384-well Plate	2 plates
---	Adhesive Plate Seal	4 seals

Quick Protocol



Additional Materials Provided by User

- Pipettes (capable of delivering between 5 to 1000 µL with appropriate tips)
- AlphaScreen™ Histidine (Nickel Chelate) Detection Kit (PerkinElmer cat# 6760619C, M or R)
- Plate reader equipped for AlphaScreen™ Detection
- Light protective cover for plate such as aluminum foil
- Plate shaker (optional)

Storage Upon receipt, store the kit at -20 °C. Under proper storage conditions, this product is stable for at least 6 months from date of receipt. Opened and reconstituted reagents are less stable refer to assay notes for additional storage information.

This product is intended for use with the Amplified Luminescent Proximity Homogenous Assay (AlphaScreen™) technology and requires the researcher to obtain donor and acceptor beads from the AlphaScreen™ Histidine (Nickel Chelate) Detection Kit, which must be purchased separately from PerkinElmer Life Sciences. A microplate reader with AlphaScreen ™ capabilities is also required.

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 present in connective tissue in higher animals. The size of HA varies from 100 kD to 10,000 kD and is responsible for different functions[1]. 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[2]. Multiple studies have shown that high serum HA levels correlates with liver disease.

Assay Design

Echelon's Hyaluronic Acid (HA) AlphaScreen™ Assay is a competitive immunoassay for in vitro HA measurement. The HA present in sample competes with the Biotin-HA for binding to the His-tag HA Detector. Therefore, the assay signal is inversely proportional to the HA amount present in the sample (Figure 1). The no-wash homogenous assay design allows assay to be completed in just 90 minutes without any additional hands-on steps between incubation. This assay is compatible with high throughput (HTS) and uses only 15 µL sample for running triplicate measurements. In addition, Echelon's HA AlphaScreen™ Assay detects HA in an independent manner regardless of HA molecular weight (see Assay Notes for details); thus, providing researchers a quick and robust in vitro HA measurement method.

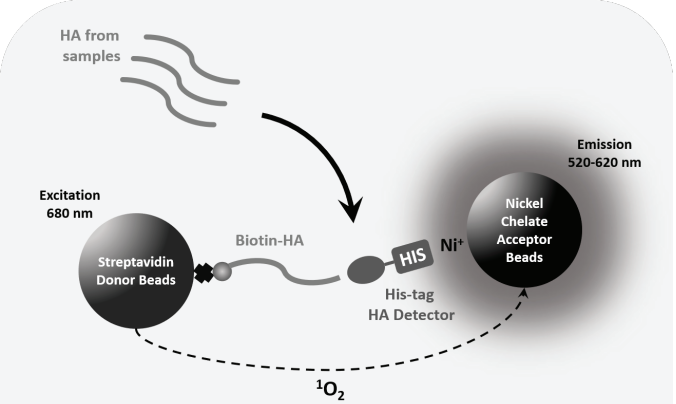
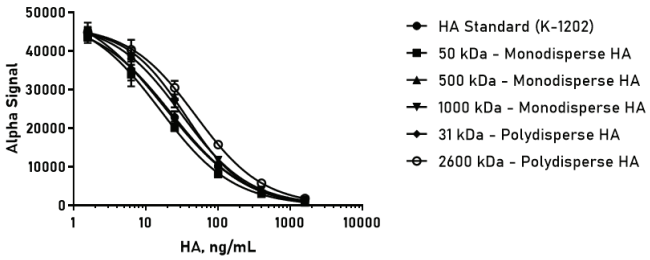


Figure 1. HA AlphaScreen™ Assay

Assay Notes

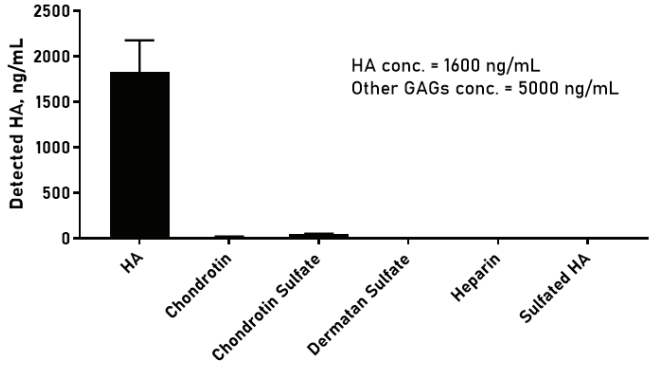
1. Use Nanopure or equivalent laboratory grade water free of contaminants.
2. Reconstituted His-tag HA Detector (Cat# K-5801) and Biotin-HA (Cat# K-5802) should be stored at -20 °C. Limit the freeze thaw cycles to avoid potential contamination.
3. Store remaining 5X Diluent (Cat# K-PBSTB) at -20 °C after use to avoid potential contamination.
4. Do not use the 10X Buffer provided in the AlphaScreen™ Histidine (Nickel Chelate) Detection Kit.
5. Refer to manufacture instruction for AlphaScreen™ Acceptor and Donor beads handling.
6. This assay is optimized using 5 µg/mL AlphaScreen™ Acceptor Beads and 20 µg/mL AlphaScreen™ Donor Beads. Changing the AlphaScreen™ beads concentrations will affect the assay signals and sensitivity.
7. To avoid high coefficient of variation within triplicates, tap plate down after addition of each reagents to ensure the added reagent droplet is at the bottom of the well.
8. If needed, the AlphaScreen™ beads can be first pre-mixed with the corresponding conjugates for easier pipetting:
   a. Combine the 5X Biotin-HA with the 5X Streptavidin AlphaScreen™ Acceptor beads at 1:1 ratio. Protect from light. Then, add 10 µL/well.
   b. Combine the 5X His-tag HA Detector with the 5X Nickel Chelate AlphaScreen™ Acceptor beads at 1:1 ratio. Then, add 10 µL/well.
9. If plate shaker is not available, tap plate to mix after all reagents are added. Plate can then be incubated without shaking but expect higher coefficient of variation.
10. Minimum of 60 minutes incubation time is needed. For best day-to-day assay consistency, 90 minutes incubation time is strongly recommended. No significant difference in assay signal and sensitivity between 60 to 180 minutes incubation.
11. This assay is optimized for measuring HA purified from biological sources.
12. Serum free media is recommended to minimize matrix effect. For cell culture media containing phenol red, a minimum of 1:5 dilution is recommended.
13. Due to matrix effects, serum/plasma samples are not recommended. If testing serum/plasma samples, a minimum of 1:20 is needed to avoid matrix effect.
14. This assay is not sensitive to HA molecular weight. No significant difference in HA detection between monodisperse and polydisperse HA with molecular weight range from 31 to 2600 kDa (Figure 2).

Figure 2. HA Molecular Weight



15. This assay does not cross react with other major glycosaminoglycans when tested at 5000 ng/mL i.e. 3.125-fold higher than the tested HA concentration (Figure 3).

Figure 3. Assay Cross Reactivity



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Assay Protocol

Please read the entire Assay Protocol and the Assay Notes before beginning experiment.

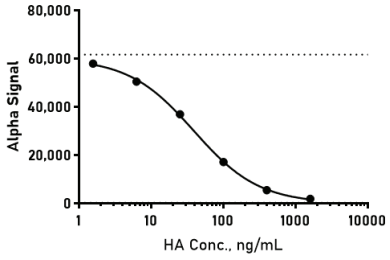
1. Equilibrate 5X Diluent (K-PBSTB) and 384-well plate(s) to room temperature before use. Keep the His-tag HA Detector (Cat# K-5801) and Biotin-HA (Cat# K-5802) on ice.
2. Prepare 1X Diluent by diluting 1-part of 5X Diluent (Cat# K-PBSTB) with 4-part of Nanopure water. Mix well. Keep at room temperature until use. Prepare just enough 1X Diluent needs to perform the current experiment, store the remaining 5X Diluent at -20 °C.
   For example, to prepare enough 1X Diluent for the entire 384-well plate, dilute 2 mL of the 5X Diluent (K-PBSTB) with 8 mL Nanopure water.
3. Reconstitute the lyophilized His-tag HA Detector (Cat# K-5801) with 30 µL Nanopure water. Pipette up and down to mix. Do not vortex. Let solution sit on ice for at least 5 minutes before further dilution to ensure complete reconstitution. This is the 500X stock solution.
4. Reconstitute the lyophilized Biotin-HA (Cat# K-5802) with 30 µL Nanopure water. Vortex to mix. Let solution sit on ice for at least 5 minutes before further dilution to ensure complete reconstitution. This is the 500X stock solution.
5. Dilute the HA Standard 2-fold by first mixing 30 µL of the HA Standard (Cat# K-1202, 3200 ng/mL) with 30 µL Nanopure water to obtain standards of 1600 ng/mL. Then, serial dilute 4-fold by mixing 20 µL of previous dilution with 60 µL Nanopure water to obtain 400, 100, 25, 6.25, 1.5625 and 0 ng/mL HA standards. (Table 1). Keep at room temperature until use.

Table 1. HA Standards Preparation for Triplicate Wells

HA Standards	Amount of Previous HA Solution Needs	Amount of Water Needs
1600 ng/mL	20 µL of HA Standard (Cat# K-1202)	20 µL
400 ng/mL	20 µL of 1600 ng/mL HA Soln.	60 µL
100 ng/mL	20 µL of 400 ng/mL HA Soln.	60 µL
25 ng/mL	20 µL of 100 ng/mL HA Soln.	60 µL
6.25 ng/mL	20 µL of 25 ng/mL HA Soln.	60 µL
1.5625 ng/mL	20 µL of 6.25 ng/mL HA Soln.	60 µL
0 ng/mL	None	60 µL

6. If necessary, dilute sample with Nanopure water. Minimum of 20 µL of samples are needed for triplicates. Keep at room temperature until use. See Assay Notes for sample dilution recommendation.
7. Prepare 5X His-tag HA Detector by diluting the 500X His-tag HA Detector stock (step 3) with the 1X Diluent (step 2) at 1:100 dilution. Keep at room temperature until use. Prepare enough 5X His-tag HA Detector needs to perform the current experiment, store the remaining 500X His-tag HA Detector stock at -20 °C.
   For example, to prepare enough 5X His-tag HA Detector for the entire 384-well plate, dilute 20 µL of the 500X His-tag Detector stock with 1.980 mL 1X Diluent. 5 µL/well of the 5X His-tag HA Detector is needed.
8. Prepare 5X Biotin-HA at by diluting the 500X Biotin-HA stock (step 4) with the 1X Diluent (step 2) at 1:100 dilution. Keep at room temperature until use. Prepare enough 5X Biotin-HA needs to perform the current experiment, store the remaining 500X Biotin-HA stock at -20 °C.
   For example, to prepare enough 5X Biotin-HA for the entire 384-well plate, dilute 20 µL of the 500X Biotin-HA stock with 1.980 mL 1X Diluent. 5 µL/well of the 5X Biotin-HA is needed.
9. Prepare 5X Nickel Chelate AlphaScreen™ Acceptor Beads at 25 µg/mL in 1X Diluent. Refer to the manufacture instruction for the Acceptor Beads stock concentration. 5 µL/well of the 5X Acceptor Beads is needed. Vortex and keep at room temperature.
10. Prepare 5X Streptavidin AlphaScreen™ Donor at 100 µg/mL in 1X Diluent. Protect from light. The AlphaScreen™ Donor Beads is sensitivity to light. Refer to the manufacture instruction for the Acceptor Beads stock concentration. 5 µL/well of the 5X Donor Beads is needed. Vortex and keep at room temperature.
11. Start assays by adding reagents sequentially as follows under dimmed light. Ensure all reagents are at room temperature before starting the assay.
    a. Add 5 µL/well of the 5X Streptavidin AlphaScreen™ Donor beads to all wells.
    b. Add 5 µL/well of the 5X Biotin-HA to all wells except blank. For blank wells, add 5 µL/well of 1X Diluent.
    c. Add 5 µL/well of the serial diluted HA Standards or samples. For blank wells, add 5 µL/well of Nanopure water.
    d. Add 5 µL/well of the 5X His-tag HA Detector except blank. For blank wells, add 5 µL/well of 1X Diluent.
    e. Add 5 µL/well of the 5X Nickel Chelate AlphaScreen™ Acceptor Beads to all wells.
12. Cover plate with seal and protect from light. Incubate at room temperature for 90 minutes with shaking.
13. After incubation, detect luminescence using plate reader equipped for AlphaScreen™ Detection.
14. Generate a best fit curve for the HA standards to interpolate the relative sample values. An example of an HA Standard Curve is shown in Figure 4 below.

Figure 4. HA Standard Curve



HA AlphaScreen™ Assay standard curve was generated using non-linear regression analysis with GraphPad Prism software. A sigmoidal does response-variable slope curve (four-parameter) analysis was utilized.

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References

1. Cowman, M. K.; Lee, H. G.; Schwertfeger, K. L.; McCarthy, J. B.; Turley, E. A., The Content and Size of Hyaluronan in Biological Fluids and Tissues. Front Immunol 2015, 6, 261.
2. Lepperdinger, G.; Fehrer, C.; Reitinger, S., Biodegradation of Hyaluronan. In Chemistry and Biology of Hyaluronan, Garg, H.; Hales, C., Eds. Elsevier Science: 2004; pp 71-82.

Related Products

Catalog #	Products
Assays and Services	
K-1200	Hyaluronan Enzyme-Linked Immunosorbent Assay (HA ELISA)
K-4800	Hyaluronic Acid Sandwich ELISA
K-6000	Hyaluronidase Activity ELISA
T-1200	Hyaluronic Acid (HA) Screening Service
Proteins & Inhibitors	
G-HA01	Versican G1 Domain
G-HA02	Biotinylated Versican G1 Domain
G-HA03	Histidine Tagged Versican G1 Domain
JK201388	4-Methylumbelliferone (HA Synthase Inhibitor)
B-0601	6-O-palmitoyl-L-ascorbic aicd (HA Synthase Inhitor)
Standards & Analogs	
HYA-LOLAD-20, HYA-HILAD, HYA-MGLAD-20	Select-HA™ Ladders
HYA-1000KEF-1, HYA-500KEF-1, HYA-601KEF-1, HYA-50KEF-1	Select-HA™
HYA-B1000-200, HYA-B500-200, HYA-B250-200, HYA-B50-200	Biotinylated Select-HA
H-025F, H250F, H-700F	BODIPY-FL HA
H-025R, H-250R, H-700R	Texas-Red HA

Please visit our website at www.echelon-inc.com for more hyaluronic acid and extracellular matrix products.

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