Hyaluronan Enzyme-Linked Immunosorbent Assay Kit (HA – ELISA)

K-1200 (96 tests)

Support: echelon@echelon-inc.com

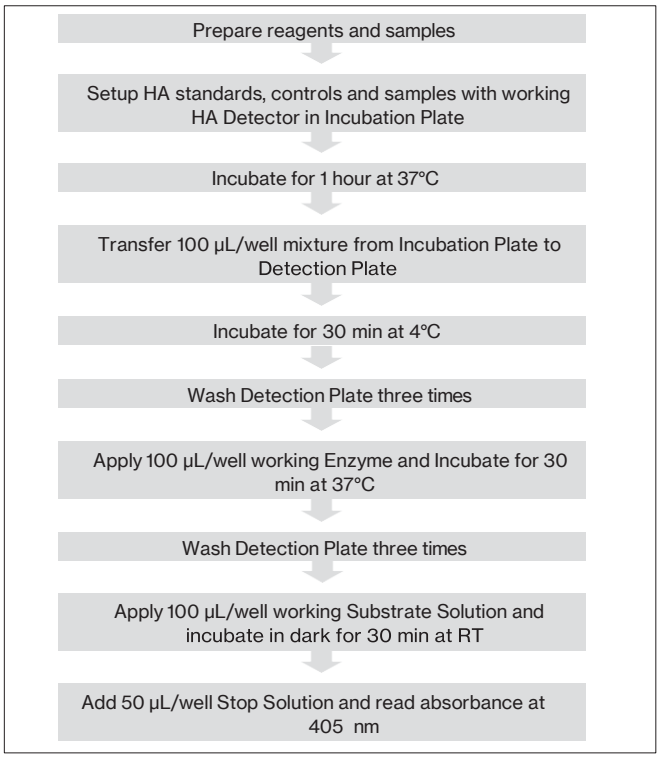
Description: The Hyaluronan Enzyme-Linked Immunosorbent Assay (HA ELISA), a competitive 96-well assay, that offers a simple, effective method for determining Hyaluronic Acid (HA) levels in human and animal biological fluids or cell supernatants.

Storage: Store kit at 4 ºC for up to six months

Materials Provided

Catalog #	Description	Quantity
K-1201	Detection Plate (12 HA-coated 8-well microwell strips w/ frame)	1 Plate
K-1202	HA Standard (3200 ng/mL HA standard solution)	1 mL
K-1203	HA Detector	< 500 µL
K-1204	Diluent (10X)	10 mL
K-PBST3	10X Wash Buffer	30 mL
K-1206	Enzyme	< 100 µL
K-1208	Substrate Buffer	12 mL
K-1209	Stop Solution	8 mL
Incubation Plate	Yellow 96-well polypropylene U-bottom	1 Plate
---	Substrate Pellet (p-Nitrophenyl Phosphate)	1 Pellet
---	Plate Seals Clear acetate sheet, 1 side Adhesive	2 Seals

Quick Protocol



Additional Materials Provided by User

- 37 ºC Incubator
- Pipettes (capable of delivering between 5 and 1,000 µL with appropriate tips)
- Multichannel pipettes
- Absorbance microplate reader capable of reading at 405 nm

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Background

Hyaluronic acid (HA) is a high molecular weight anionic polysaccharide (1,000-10,000 kD) composed of repeating disaccharides of β(1-4)glucuronic acid and β(1-3)N-acetylglucosamine and is one of several glycosaminoglycan (GAG) components of the extracellular matrix (ECM) of connective tissue (1). Each disaccharide dimer is referred to as one unit and has an approximate molecular weight (MW) of 450 D. Depending on the tissue source, the polymer can consist of 2,000 to 25,000 units (2). HA is an extremely large molecule not necessarily in its molecular weight but in the space that it occupies in solution which lends to its remarkable viscoelastic properties, lending to its importance in joint lubrication (3).

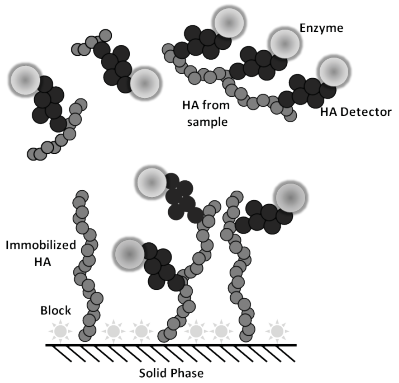
Several functions of HA have been described including influencing the hydration and physical properties of tissues; and its ECM (extra- cellular matrix) interactions which affect tissue structure, assembly and facilitation of cell movement and behavior.

Free HA is transported from the lymph to the circulation with an estimated half-life in serum of 2-5 minutes. HA is taken up by the liver in sinusoidal endothelial cells (90%) and the kidneys (10%) where it is degraded and recycled (4,5). Many chronic liver diseases, including infection (hepatitis B or C), toxicity (alcohol and drugs), genetic (hemochromatosis), autoimmunity, and malignancy, result in liver inflammation which can progress to liver fibrosis and cirrhosis (6). Each of these cause impairments of liver function and result in a rapid increase in circulating HA levels (4). Data indicates a relationship between HA levels, local inflammation and severity of disease (6).

Recent publications have shown that HA levels in urine are indicative of bladder cancer, that HA levels are directly correlated to liver disease and suggests enhanced breakdown of HA in the lungs of patients with chronic obstructive pulmonary disease (COPD). In addition, serum levels of HA have been found to be elevated in patients with rheumatoid arthritis (7).

Assay Design

The HA-ELISA is a quantitative enzyme-linked immunoassay designed for the in vitro measurement of HA levels in human or animal biological fluids (blood, serum, urine, diffusate, synovial fluid). This simple protocol requires 3 hours.



The HA-ELISA is a competitive ELISA in which the colorimetric signal is inversely proportional to the amount of HA present in the sample. Samples to be assayed are first mixed with the HA Detector, and then added to the HA ELISA plate for competitive binding. An enzyme-linked antibody and colorimetric detection is used to detect the HA detector bound to the plate. The concentration of HA in the sample is determined using a standard curve of known amounts of HA. The enzyme /substrate system is a colorimetric assay comprised of alkaline phosphatase / pNPP phosphatase substrate. It should be read at 405 nm.

The size of HA polymers is variable depending on tissue source. However, the sensitivity of the HA ELISA does not depend on the MW of the HA molecule except in the lower MW range (<25 dimers). The HA ELISA works best with HA molecules that are greater than 25 repeating units (dimers) to determine the relative concentration of HA independent of MW.

Assay Notes

1. We suggest the HA Standard dilution series be run in triplicate for best results.
2. It is recommended that serum and plasma samples be analyzed with no dilution or at a maximum 2-fold dilution in the provided Diluent.
3. When analyzing biologic samples, we advise running a known normal (low) HA sample and a disease (high) HA sample in conjunction with your unknown samples. These will serve as positive and negative controls to distinguish between normal healthy samples and disease samples.
4. Reference HA Values: Normal HA levels in serum from healthy blood donors are less than 120 ng/mL. Serum HA levels are elevated in several disease states including hepatitis (greater than 160 ng/mL) and cirrhosis (greater than 250ng/mL).
   ** Important Note: The above values are the suggested values based on literature observations. At times, the values measured using the Echelon Hyaluronic Acid ELISA assay have been 2-3-fold higher than those expected from the literature. As a result, we strongly advise users to utilize known reference samples indicative of both normal and disease states to establish relevant Hyaluronic acid levels. This will allow the user to differentiate between normal and disease state Hyaluronic Acid samples in a qualitative fashion.**

Protocols

Note: all reagents of the kit, once at working concentration, can be stored at -20 ºC for up to 2 months and can handle at least 1 freeze/defrost cycle. Reagents at working concentration, except the working Substrate Solution, can also be stored at 4 ºC for 2 weeks. The working Substrate Solution can be used for up to 48 hours when stored at 4 ºC. Additional Substrate Buffer (K-1208) and pellet can be purchased under catalog number K-1208T.

Reagent Preparation

1. 1X Diluent: The Diluent is supplied as a 10X concentrated solution (K-1204). Dilute the required amount to a 1X working Diluent with dH2O prior to beginning assay. Typically, around 30-35 mL of Diluent

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is required to run a full assay. For 30 mL of 1X Diluent, add 3 mL of the 10X Diluent to 27 mL of dH2O.

2. HA Standards: Gently mix HA standard. Make 2-fold serial dilutions of the HA Standard (K-1202) using the Diluent to obtain standards of 1600, 800, 400, 200, 100, and 50 ng/mL (Standards may be diluted in the incubation plate, following the diagram below).
3. Working Detector: Dilute Detector (K-1203) by adding 6 mL Diluent. The volume of the Detector provided is small and the bottle may appear empty.
4. Working Enzyme: Dilute Enzyme (K-1206) by adding 12 mL Diluent. The volume of Enzyme provided is small and the bottle may appear empty.
5. 1X Wash Buffer: Add the 10X Wash Buffer (K-PBST3) to 270 mL deionized water for a 1X Wash Buffer solution. Final volume = 300 mL
6. Working Substrate Solution: Dissolve Substrate Pellet in 12 mL Substrate Buffer (K-1208).

Assay Procedure

7. Set up the Incubation Plate (yellow U-bottom plate) as illustrated. Each well should contain 150 µL.
    a. Add 100 µL of Standards and samples into corresponding wells.
    b. Add 150 µL of Diluent to the Blank control wells and 100 µL of Diluent to the Zero HA control wells.
    c. Add 50 µL of working HA Detector to all wells except the Blank control wells.
8. Mix the plate gently, cover with plate seal and incubate for one hour at 37 ºC.
9. Following the incubation step, transfer 100 µL of controls and samples from the Incubation Plate to the corresponding wells of the Detection Plate (K-1201). This is easily accomplished with a multi-channel pipettor.
10. Once the transfer is complete, mix the Detection Plate by gently tapping. Cover with a plate seal and incubate at 4 ºC for 30 minutes.
11. Shake out the solution from the Detection Plate. Wash the plate 3 times with 200 µL of 1X Wash Buffer. Ensure all wash buffer is removed from the plate by inverting the plate and blotting it out on absorbent paper.
12. Add 100 µL of working Enzyme (K-1206) to each well of the Detection Plate.
13. Mix the Detection Plate gently, cover with plate seal and incubate at 37 ºC for 30 minutes.
14. Repeat wash step #11.
15. Add 100 µL working Substrate Solution (K-1208) to each well of the Detection Plate. Incubate plate in dark at room temperature for 30 min.
16. Stop the reaction by adding 50 µL Stop Solution (K-1209) to each well. Read the detection plate to measure the absorbance at 405 nm.

Quantification of Samples

Generate a best fit curve for the standards to interpolate relative sample values. (See figure 1.)

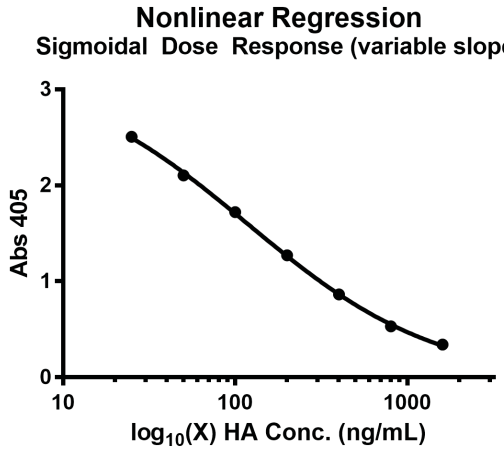

Figure 1: HA competitive ELISA standard curve was generated using non-linear regression analysis with GraphPad Prism software. A sigmoidal dose response-variable slope curve (four-parameter) analysis was utilized.

Suggested Incubation Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank Contro	Blank Contro	Sample #1	Sample #1	Sample #9	Sample #9	Sample #17	Sample #17	Sample #25	Sample #25	Sample #33	Sample #33
B	1600 ng/mL	1600 ng/mL	Sample #2	Sample #2	Sample #10	Sample #10	Sample #18	Sample #18	Sample #26	Sample #26	Sample #34	Sample #34
C	800 ng/mL	800 ng/mL	Sample #3	Sample #3	Sample #11	Sample #11	Sample #19	Sample #19	Sample #27	Sample #27	Sample #35	Sample #35
D	400 ng/mL	400 ng/mL	Sample #4	Sample #4	Sample #12	Sample #12	Sample #20	Sample #20	Sample #28	Sample #28	Sample #36	Sample #36
E	200 ng/mL	200 ng/mL	Sample #5	Sample #5	Sample #13	Sample #13	Sample #21	Sample #21	Sample #29	Sample #29	Sample #37	Sample #37
F	100 ng/mL	100 ng/mL	Sample #6	Sample #6	Sample #14	Sample #14	Sample #22	Sample #22	Sample #30	Sample #30	Sample #38	Sample #38
G	50 ng/mL	50 ng/mL	Sample #7	Sample #7	Sample #15	Sample #15	Sample #23	Sample #23	Sample #31	Sample #31	Sample #39	Sample #39
H	Zero HA	Zero HA	Sample #8	Sample #8	Sample #16	Sample #16	Sample #24	Sample #24	Sample #32	Sample #32	Sample #40	Sample #40

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Related Products

Products	Catalog Number
Assays and Reagents	
HA Sandwich ELISA	K-4800
HA AlphaScreen Assay	K-5800
Hyaluronidase Activity ELISA	K-6000
Binding Proteins	
Versican G1 Domain	G-HA01
Biotinylated Versican G1 Domain	G-HA02
His Tagged Versican G1 Domain	G-HA03
Select HA	
Select-HA LoLadder	HYA-LOLAD
Select-HA HiLadder	HYA-HILAD-20
Select-HA Mega Ladder	HYA-MGLAD-20

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