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Hyaluronan Immunoassay

Catalog Number:SEKH-0509

For the quantitative determination of human Hyaluronan concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY:To assess the linearity of the assay, three samples were spiked with high concentrations of Hyaluronan in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	104	108
	Range(%)	93–114	98–117
1:4	Average% of Expected	105	109
	Range(%)	96–113	99–118
1:8	Average% of Expected	103	104
	Range(%)	96–109	93–114
1:16	Average% of Expected	102	103
	Range(%)	93–107	97–108

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 0.35ng/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human Hyaluronan. The factors listed below were prepared at 400ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
Aggrecan (aa 20-675)	HABP1/C1qBP	CD44
Aggrecan (aa 1346-2204)	Layilin	
CD44H	LYVE-1	
CD44v2		
CD44v3-10		
HABP1/C1qBP		
Layilin		

REPEATABILITY:The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of Hyaluronan spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Hyaluronan in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	98	89–95
Cell culture supernatants	103	94–111

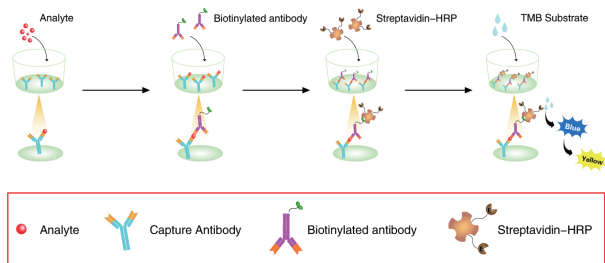
BACKGROUND

Hyaluronan, also known as hyaluronic acid (HA) or sodium hyaluronate, is a naturally occurring linear polymer. Hyaluronan is a glycosaminoglycan (GAG) that is ubiquitously present in the extracellular matrix of all vertebrates and is also present in the capsule of some strains of Streptococci. Mammalian hyaluronan is synthesized by one of three distinct hyaluronan synthases (HAS1, 2, and 3), which produce HA polymers with different chain lengths and differ in their rates of synthesis. Whereas high molecular weight HA (> 500 kDa) is anti-angiogenic, anti-inflammatory and immunosuppressive, low molecular weight HA (10 - 500 kDa) is highly angiogenic and pro-inflammatory. HA oligomers are anti-apoptotic and upregulate heat shock protein expression. Functionally, hyaluronan molecules are important for the maintenance of a highly hydrated extracellular matrix in tissues, which is involved in cell adhesion and supports cell migration.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Hyaluronan has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Hyaluronan present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for Hyaluronan is added to detect the captured Hyaluronan protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

DESCRIPTION



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

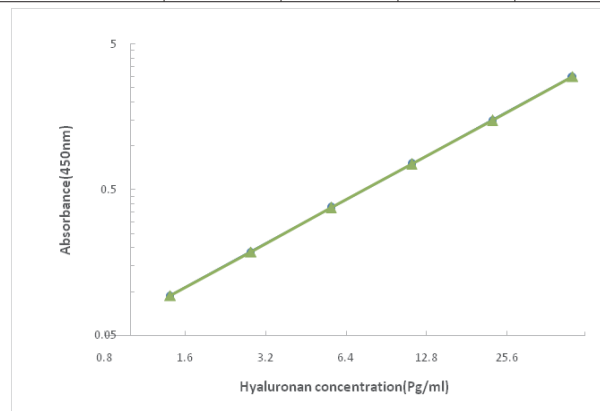
DESCRIPTION

determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by 5 the dilution factor.

This standard curve is provided for demonstration only. A standard

Typical data using the Hyaluronan ELISA

Standardized (ng/ml)	OD.	OD.	Average	Corrected
0	0.048	0.041	0.045	-
0.70	0.185	0.187	0.186	0.141
1.4	0.269	0.272	0.270	0.226
3	0.400	0.404	0.402	0.357
5.625	0.635	0.642	0.638	0.594
11.25	1.031	1.042	1.036	0.992
22.5	1.678	1.697	1.687	1.643
45	2.734	2.765	2.749	2.705



Representative standard curve for Hyaluronan ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash three times before assay.



Add 100µl standard or samples to each well, incubate 120 minutes, at room temperature (25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human Hyaluronan antibody to each well, incubate 60 minutes, at room temperature (25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes, at room temperature (25±2°C).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 30 minutes, at room temperature (25±2°C). Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the Hyaluronan concentrations versus the log of the O.D. and the best fit line can be

KIT COMPONENTS & STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells x 12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized, 45ng/ml upon reconstitution	2 vials	Aliquot and Store at 2-8°C ** for six months
Concentrated Biotin-Conjugated antibody (100X) - 120 ul/vial	1 vial	Store at 2-8°C ***for six months
Concentrated Streptavidin-HRP solution (100X) - 120 ul/vial	1 vial	Store at 2-8°C ***for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C ***for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C ***for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C ***for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent.** This reconstitution produces a stock solution of 45ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 μL of Standard/Sample Diluent into the remaining tubes. Use the high standard to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 45ng/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 ng/mL).

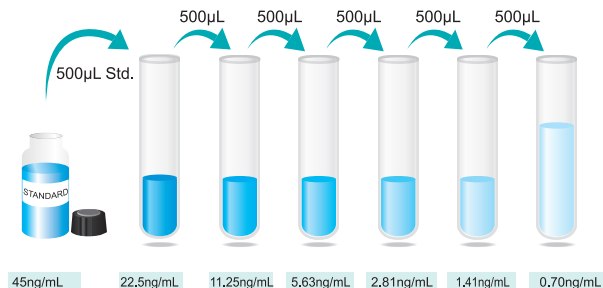
*If you do not run out of re-melting standard, store it at -20°C . Diluted standard shall not be reused.

4. **Working solution of Biotin-Conjugate anti-human Hyaluronan antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

*The working solution should be used within one day after dilution.

5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

*The working solution should be used within one day after dilution.



Preparation of Hyaluronan standard dilutions