Hyaluronan Quantification Kit

(ELISA-like assay for HA / Hyaluronic Acid)

Cat. No. AMS.CSR-HA-96KIT

Updated on Jan. 6th, 2017

【1-1】Background

Hyaluronan (HA) is an unbranched glycosaminoglycan composed of repeating disaccharide units of D-glucronic acid and N-acetyl-D-glucosamine. HA is abundant in synovial fluid, skin, umbilical cord, and vitreous bodies. HA is a prominent component of the extracellular matrix and contributes to tissue water retention and to cellular growth, differentiation, and migration. HA exists in the body in a wide range of molecular weights (MW) derived from several distinct HA synthases and degradation enzymes. For example, the average molecular weights of HA in synovial fluid, blood and urine are 4-6 MDa, 100-300 kDa and less 10 kDa, respectively.

This product is a competitive ELISA-like kit using an HA binding protein optimized to quantify HA polymers of average molecular weight greater than 7.4 kDa in samples such as serum, plasma and culture supernatant.

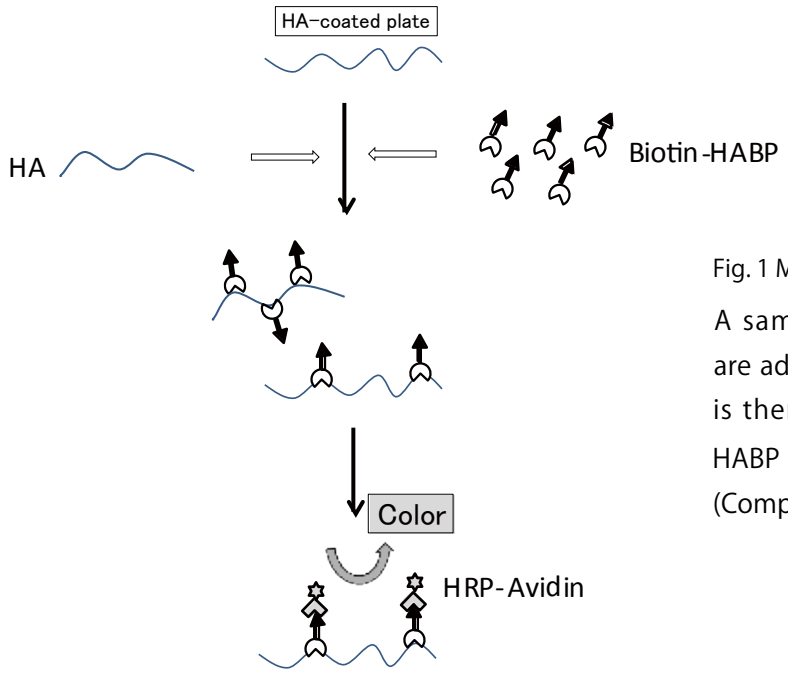


Fig. 1 Measurement principle

A sample containing HA and Biotin-HABP are added to an HA-coated plate. HRP-Avidin is then added, and the HA binding Biotin-HABP is detected by chromophoric substrate (Competition Method).

For research use only, Not for diagnostic use.
Please read this manual thoroughly before use.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

【1-2】Features

- Hardly getting affected by the molecular size of HA.
- Measurable the HA concentrations accurately in blood serum, plazma and supernatant of which the molecular weight is above 7.4 kDa.
- Measuring range of HA concentration is between 3.13 ～ 200 ng/mL.

【1-3】Kit Component

Materials Provided

Description	Volume	Quantity
96 well plate	8 x 12 wells	1
HA Coating Solution	11 mL	1
Wash Buffer (20X)	50 mL	1
Sample Buffer (2X) *	50 mL	1
Blocking Buffer (2X) *	11 mL	1
HA Standard (10μg/mL) *	0.1 mL	1
Biotin-HABP *	10 ～ 40 μL	1
HRP-Avidin (100X) *	0.2 mL	1
Substrate Solution A	1 mL	1
Substrate Solution B	10 mL	1
Stop Solution	11 mL	1
Plate Seals	5 mL	2

*：containing 0.025 ～ 0.05% Proclin 300

Strage: ≦－ 20℃

Expiration Date: labeled on the box

Materials Required But Not Provided

1. Microplate reader capable of measuring absorbance at 450 nm
2. Horizontal orbital microplate shaker
3. Multichannel pipettes
4. Pipettes and pipette tips
5. Paper towels
6. Bottles and test tubes
7. Graduated cylinder (500 mL or 1000 mL)
8. Deionized or distilled water

【2】Reagent Preparation

Reagents should be mixed well before use.

1. 1X Wash Buffer

   Add 50 mL of Wash Buffer (20X) to 950 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer. If crystals have formed in Wash Buffer (20X), warm to around 37℃ and dissolve completely.

2. 1X Sample Buffer

   Add 50 mL of Sample Buffer (2X) to 50 mL of deionized or distilled water to prepare 100 mL of 1X Sample Buffer.

3. 1X Blocking Buffer

   Add 10 mL of Blocking Buffer (2X) to 10 mL of deionized or distilled water to prepare 20 mL of 1X Blocking Buffer.

4. 1X Biotin-HABP

   Add 6 mL of 1X Sample Buffer to an appropriate tube (tube A). Take 0.5 mL of 1X Sample Buffer from tube A to Biotin-HABP tube and mix well. Return all the solution in Biotin-HABP tube to tube A and mix well.

5. HA Standard

   Prepare eight 1.5 mL tubes (No.1 ～ No.8). Pipette 980 µL of 1X Sample Buffer to No.1 tube, and 500 µL of 1X Sample Buffer to No.2 ～ No.8 tubes. Pipette 20 µL of HA Standard (10 µg/mL) to No.1 tube, and mix well (200 ng/mL). Pipette 500 µL of HA Standard (No.1 tube, 200 ng/mL) to No.2 tube, and make 2-fold serial dilutions to No.7 tube in a similar manner. Mix each tube well before the next transfer.

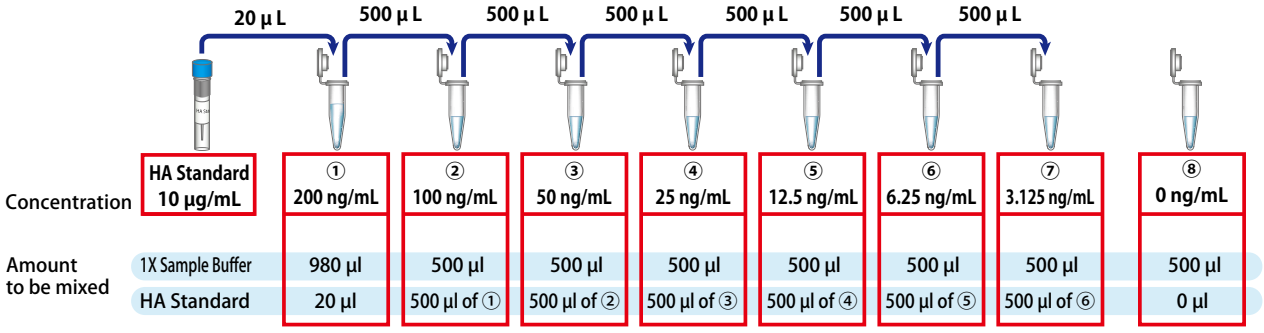


Fig. 2. Dilution of HA Standard

6. Preparation of test samples

   Samples should be diluted appropriately with 1X Sample Buffer.

7. 1X HRP-Avidin

   Pipette a 100 µL of HRP-Avidin (100X) to 9.9 mL of 1X Sample Buffer to prepare 10 mL of 1X HRP-Avidin.

8. Substrate Solution

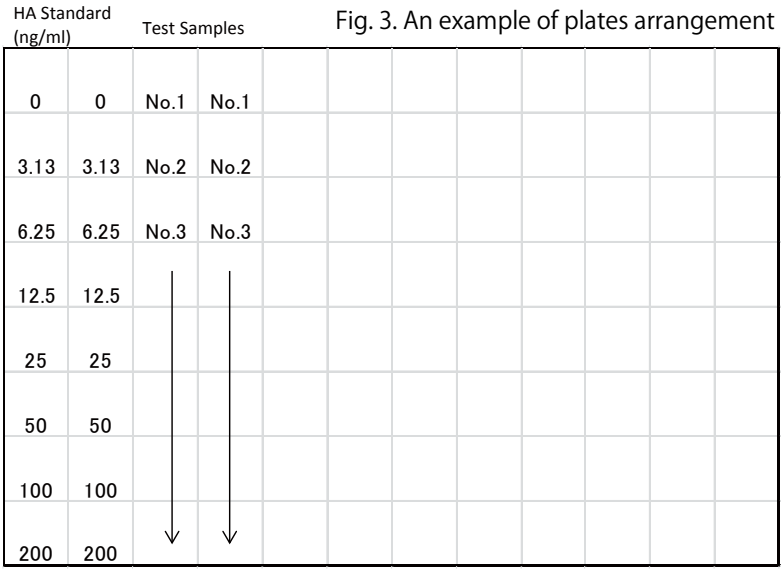
   Mix 1 mL of Substrate Solution A and 10mL of Substrate Solution B to prepare 11 mL of Substrate Solution within 15 minutes of use. Protect from light. Please use up all the prepared solution and dispose the remainder.

【3-1】Assay Procedure

Bring all reagents and test samples to room temperature before beginning test.

1. Vortex HA Coating Solution bottle well before use. Add 100 µL of HA Coating Solution to each well. Cover with the plate seal provided. Incubate for 1 hour at room temperature.
2. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µL of Wash Buffer using a multi-channel pipette. Invert the plate and blot it against paper towel.
3. Add 200 µL of 1X Blocking Buffer to each well. Cover with the plate seal and incubate for 30 minutes at room temperature.
4. Discard the solution and wash 1 times with 300 µL of 1X Wash Buffer.
5. Add 50 µL of each standard and sample to appropriate well (n=2).
6. Add 50 µL of 1X Biotin-HABP to all the wells. Cover with the plate seal and mix for 30 seconds using microplate shaker. Incubate for 1 hour at room temperature.
7. Discard the solution and wash 4 times with 300 µL of 1X Wash Buffer.
8. Add 100 µL of 1X HRP-Avidin to each well. Cover with the plate seal and incubate for 1 hour at room temperature.
9. Discard the solution and wash 4 times with 300 µL of 1X Wash Buffer.
10. Add 100 µL of Substrate Solution to each well. Incubate for 20-30 minutes in the dark.
11. Add 100 µL of Stop Solution to each well. Read the absorbance at 450 nm using a microplate reader, immediately.
12. Plot the standard curve with standard concentration and absorbance, and calculate the concentration of HA in the samples.

Fig. 3. An example of plates arrangement



【3-2】Precautions for Use

1. Avoid repeated freeze-thaw cycles of samples and reagents.
2. Reagents except subtrate solution should be prepared at time of use and may be stored for up to 1 week at 2-8℃ . For Substrate Solution, it should be used immediately. Do not keep it once prepared.
3. Change pipette tips between the preparation and addition of each standard and test sample to avoid cross-contamination.
4. The standard curve must be run with each assay.
5. Do not mix and use different lots of reagents.

【4】Measuring Range

The measuring range of HA using Hyaluronan Quantification Kit is 3.13 ～ 200 ng/mL (Fig. 4).

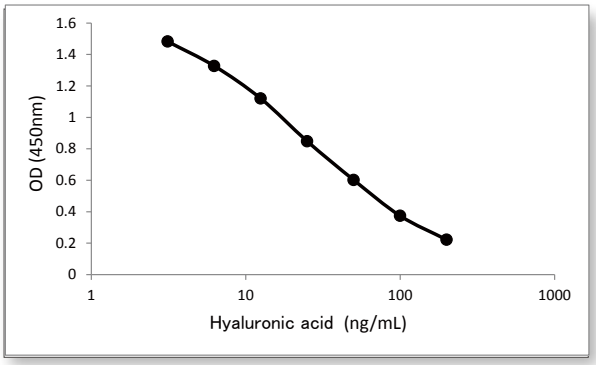


Fig. 4. Typical calibration curve

【5】Concentrations of HA in Serum and Plasma

The concentration of HA in normal human and animal sample is summarized in Table 1.

Samples	n	Mean (ng/mL)
Human plasma	30	18.1
Human serum	3	29.2
Rabbit serum	8	37.1
Rat serum	2	213.3

Table1. HA contents in plasma and serum

【6】Specificity

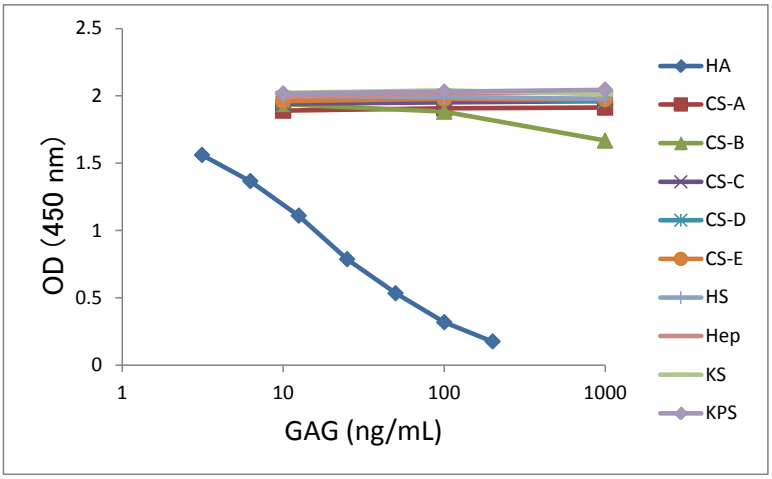


Fig. 5. The cross-reactivity with various kinds of GAGs

The cross-reactivity with various kinds of glycosaminoglycans (GAGs) was evaluated using Hyaluronan Quantification Kit (Fig.5). This kit did not show the cross-reactivity with chondroitin sulfate-A (CS-A), CS-C, CS-D, CS-E, heparan sulfate (HS), heparin (Hep), keratan sulfate (KS) and keratan polysulfate (KPS) at the concentrations of up to 1000 ng/mL. A weak reactivity was shown in high concentration of CS-B (dermatan sulfate).

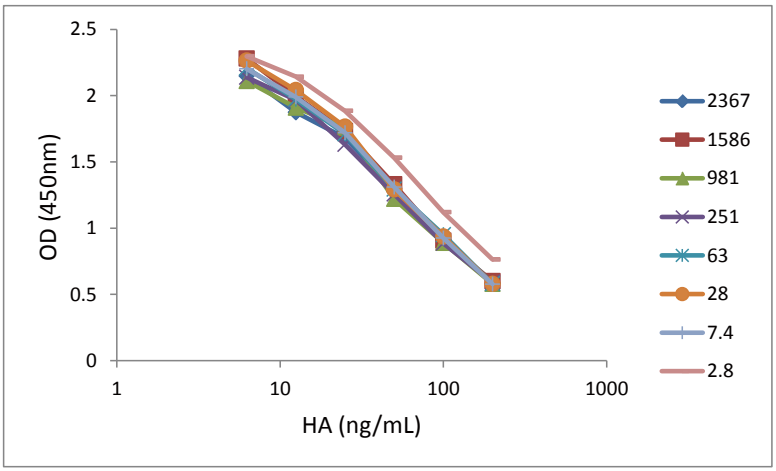


Fig. 6. The reactivity with various molecular weights of HA
(HA with molecular weights of from 2.8 to 2367 kDa were compared)

The reactivity with various molecular weights of HA was evaluated using Hyaluronan Quantification Kit (Fig. 6). When using HA with molecular weights of from 2.8 to 2367 kDa, this kit reacted with molecular weights of over 7.4kDa HA in a similar manner. HA with a molecular weight of 2.8kDa showed a slightly weaker reactivity (Fig. 6).

【Ⅶ】Various Tests

Intra-assay Precision

Table 2. Intra-assay Precision

	Mean	SD	CV(%)
Sample L	8.9	0.6	7.1
Sample M	38.1	0.9	2.3
Sample H	149.8	3.6	2.4

unit: ng/mL, SD: standard deviation, CV: coefficient of variation

Three samples of known concentration (Sample L, M, H) were tested eight times in duplicate on one plate to assess precision within an assay. The CVs were less than 10% (Table 2).

Inter-assay Precision

Table 3. Inter-assay Precision

	M 1	M 2	M 3	M 4	Mean	SD	CV(%)
Sample L	8.9	8.3	10.4	10.2	**9.5**	**0.9**	**9.2**
Sample M	38.1	34.5	41.2	39.7	**38.4**	**2.5**	**6.5**
Sample H	149.8	148.7	154.6	151.3	**151.1**	**2.2**	**1.5**

unit: ng/mL, M: measurement, SD: standard deviation, CV: coefficient of variation

Three samples of known concentration (Sample L, M, H) were tested four times in duplicate on different days to assess precision between assays. The CVs were less than 10% (Table 3).

Recovery

	Spike	Estimated	Obserbed	Recovery%
No.1	0		35.4	
	12.5	47.9	47.4	**98.9**
	25	60.4	61.9	**102.4**
	50	85.4	89.6	**104.9**
No.2	0		17.8	
	12.5	30.3	31.8	**104.8**
	25	42.8	44.9	**104.8**
	50	67.8	72.5	**106.9**
No.3	0		7.5	
	12.5	20.0	21.3	**106.0**
	25	32.5	34.6	**106.2**
	50	57.5	60.1	**104.4**

Table 4. Recovery

Unit: ng/mL

The recovery of HA spiked to three different levels in three different serum samples throughout the range of assay was evaluated. The value of recovery was between 98.9 - 106.9% (Table 4).

Linearity

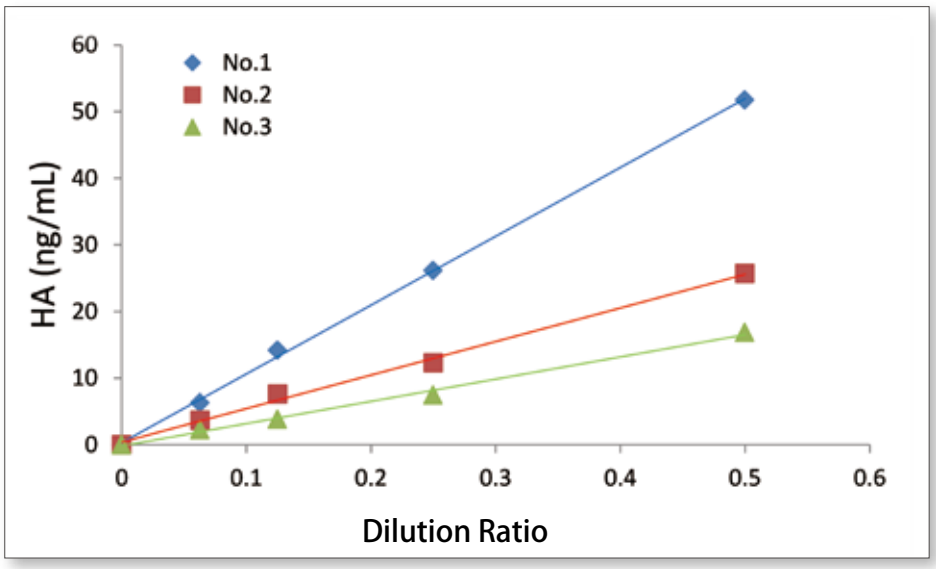


Fig. 7. Linearity

Linearity was evaluated using serially diluted three human sera (No.1 - 3). When samples were diluted with sample buffer (dilution ratio 0.0625 - 0.5), good correlation coefficients (r>0.998) were observed (Fig. 6).

【8】References

1. Haserodt S et al., A comparison of the sensitivity, specificity, and molecular weight accuracy of three different commercially available Hyaluronan ELISA-like assays. Glycobiology. 21(2):175-183. 2011.
2. Maeda H et al., A competitive enzyme-linked immunosorbent assay-like method for the measurement of urinary hyaluronan. Biosci Biotechnol Biochem, 63(5):892-895, 1999.

AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85