Hyaluronan Sandwich Assay Kit

Catalog # 6049

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Sandwich ELISA to quantify high molecular weight Hyaluronan (HA) (>150 kDa)
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	16 - 1000 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (3.1-5.9%)/Inter-Assay (7.8-9.8%)/Spiking Test (96-109%)
NOTES:	Assays need to be performed at 4°C for optimal sensitivity

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

Hyaluronan Sandwich Assay Kit

Catalog # 6049

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Hyaluronan (HA: also called Hyaluronic acid) is a non-sulfated, unbranched glycosaminoglycan (GAG) composed of repeating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine (1). Nearly all vertebrate cells can produce HA, with its expression closely linked to processes such as tissue expansion and cell movement (2). HA is primarily found in the extracellular and pericellular matrices, though it can also be present intracellularly. Its key biological roles include maintaining the elastoviscosity of fluid connective tissues such as joint synovial fluid and the vitreous humor in the eye, regulating tissue hydration and water transport, and organizing proteoglycans in the extracellular matrix. Additionally, HA is involved in receptor-mediated processes like cell detachment, mitosis, migration, tumor progression and metastasis, and inflammation. It also plays a vital role in binding water and lubricating movable parts of the body, such as joints and muscles (3). HA receptors on effector cells interact with HA and are involved in HA-related cell behavior and uptake (4).

HA is synthesized by at least three distinct, apparently functionally redundant synthases (HAS1, HAS2, and HAS3) in the plasma membrane from which it is released into the extracellular matrix (ECM) (5). It is degraded by hyaluronidases, which cleave HA into smaller fragments that exhibit different biological activities (5). Changes in HA levels and molecular weight distribution can serve as biomarkers for various diseases, including cancer, rheumatoid arthritis, and liver fibrosis (6). High molecular weight (HMW) HA (> 500 kDa) is anti-angiogenic, anti-inflammatory, and immunosuppressive, whereas low molecular weight (LMW) HA (10 - 500 kDa) is highly angiogenic and pro-inflammatory (7).

In ECM studies, particularly scaffold preparation, components such as GAGs and collagen must be validated. The four primary groups of GAGs are classified based on their core disaccharide units and include heparin/heparan sulfate, chondroitin sulfate/dermatan sulfate, keratan sulfate, and hyaluronic acid (8). Commonly used GAG assays, such as those utilizing 1,9-dimethylmethylene blue (DMMB), are only effective for detecting sulfated GAGs and cannot specifically identify HA. This limitation makes the accurate determination of HA difficult when evaluating scaffolds. However, HA is present across all vertebrates with a uniform structure, regardless of the species or tissue that produces it; this uniformity makes it challenging to generate antibodies against this GAG. As a result, HA-binding proteins (HABP) are commonly used as probes for detecting HA (9).

Chondrex, Inc. offers an HA Sandwich Assay kit (Cat # 6049) that utilizes recombinant HABPs and a sandwich ELISA system for measuring HA levels in samples such as cell culture media and serum. This kit is optimized for detecting higher molecular weight HA (>150 kDa). For detecting lower molecular weight HA (<150 kDa), a competitive assay kit (Cat # 6048) is available (10). In addition, Chondrex, Inc. also offers a GAG kit (Cat # 6022) can be used to detect coexisting sulfated components in ECM.

This kit contains enough reagents to measure 40 samples in duplicate together with standards. Please visit www.chondrex.com or contact support@chondrex.com for more information.

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Hyaluronan (HA) (60491)	1 vial	1000 ng, lyophilized	-20°C
Hyaluronan Binding Protein (HABP) (60492)	1 vial	200 µl, 50 µg/ml	-20°C
Biotinylated-HABP (60493)	1 vial	100 µl	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	2 bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

NOTE 8: All steps should be performed at 4ºC, except for TMB color development, which should be conducted at room temperature to ensure optimal assay sensitivity.

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

ASSAY OUTLINE

Add 100 µl of diluted HABP into wells

Incubate at 4°C overnight.
Wash plate.

Add 100 µl of Solution B

Incubate at 4°C for 1 hour.
Wash plate.

Add 100 µl of diluted standards and samples into wells

Incubate at 4°C for 1 hour.
Wash plate.

Add 100 µl of diluted Biotin-HABP

Incubate at 4°C for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase

Incubate at 4°C for 30 minutes.
Wash plate.

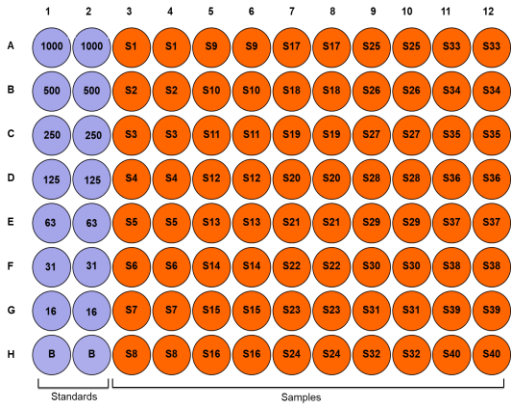
Add 100 µl of TMB solution

Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

Read plates at 450 nm/630 nm

PLATE MAPPING



© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

ASSAY PROCEDURE

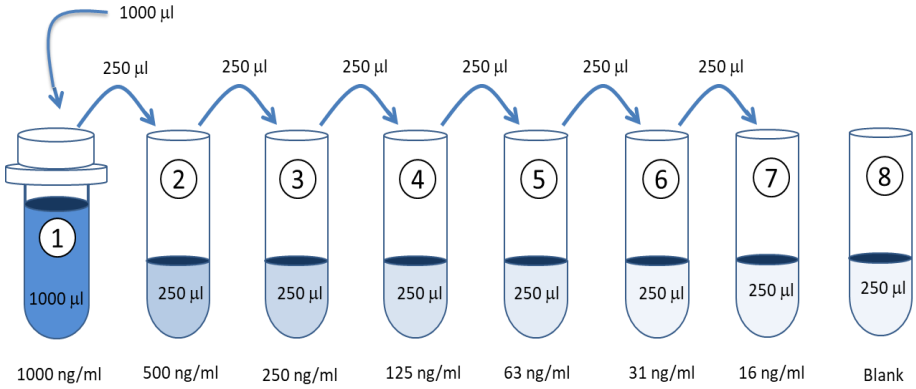
1. **Add HABP Solution:** Dilute one vial of HABP with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of HABP solution to each well and incubate at 4°C overnight. Any leftover HABP Stock Solution may be stored at -20°C for future assays.

Strip #	HABP (µl)	Solution A (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

2. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

3. **Add Blocking Buffer**: Add 100 µl of Solution B to each well and incubate at 4°C for 1 hour.

4. **Prepare Standard Dilutions**: The recommended standard range is 16 - 1000 ng/ml. Dissolve one vial of Standard (1000 ng/vial) in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) and keep it as standard stock. Then serially dilute it with Solution B. For example, mix 250 µl of the 1000 ng/ml solution with an equal volume of Solution B to make a 500 ng/ml solution, and then repeat it five more times for 250, 125, 63, 31 and 16 ng/ml standard solutions.



5. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution B depending on the estimated HA level in the samples. Two or three different sample dilutions are recommended if the HA levels in the samples are unknown.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Standards and Samples**: Add 100 µl of standards, Solution B (blank), and samples to wells in duplicate. Incubate at 4°C for 1 hour.

8. **Prepare Biotinylated-HABP**: Dilute one vial of Biotinylated-HABP in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). Alternatively, dilute according to the table below. Any leftover Biotinylated-HABP Stock Solution may be stored at -20°C for future assays.

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

Strip #	Biotin-HABP (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

9. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

10. **Add Biotinylated-HABP Solution**: Add 100 µl of Biotinylated-HABP solution to each well and incubate at 4°C for 1 hour.

11. **Prepare Streptavidin Peroxidase**: Dilute one vial of streptavidin peroxidase in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). Alternatively, dilute according to the table below. Any leftover streptavidin peroxidase stock solution may be stored at -20°C for future assays.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

12. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

13. **Add Streptavidin Peroxidase Solution**: Add 100 µl of streptavidin peroxidase solution to each well and incubate at 4°C for 30 minutes.

14. **Prepare TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, dilute 1 vial of TMB with 10 ml of Chromogen Dilution Buffer. Alternatively, dilute according to the table below.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

15. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

16. **Add TMB Solution**: Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

17. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) in each well.

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

18. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATING RESULTS

1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of HA standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 16 to 1000 ng/ml.

4. The ng/ml of HA in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the HA concentration (ng/ml) in the original test samples.

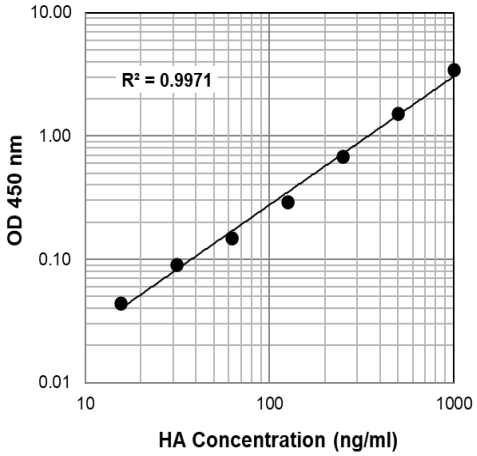


Figure 1 - A Typical Standard Curve for the HA Sandwich Assay Kit

VALIDATION DATA

Table 1 - Reproducibility Data for the HA Sandwich Assay Kit

Test	31 ng/ml	125 ng/ml	500 ng/ml
Intra-Assay CV (%)	4.7	3.1	5.9
Inter-Assay CV (%)	7.9	9.8	7.8
Spike Test* (%)	98%	96%	109%

*Known amounts of HA were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) to assay HA by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

REFERENCES

1. I. Hargittai, M. Hargittai. Molecular structure of hyaluronan: an introduction. *Struct Chem* **19**, 697-717 (2008).

2. V. Hascal, J. Esko. "Hyaluronan" in *Essentials of Glycobiology* (Cold Spring Harbor Laboratory Press, ed. 3, 2017).

3. J. Necas, L. Bartosikova, P. Brauner, J. Kolar. Hyaluronic acid (hyaluronan): a review. *Vet Med (Praha)* **53(8)**, 397-411 (2008).

4. B. Toole. Hyaluronan and its binding proteins, the hyaladherins. *Curr Opin Cell Biol* **2(5)**, 839-44 (1990).

5. S. Garantziotis, R. Savani. Hyaluronan biology: A complex balancing act of structure, function, location and context. *Matrix Biology* **78**, 1-10 (2019).

6. A. Engström-Lauren. "Changes in hyaluronan concentration in tissues and body fluids in disease states" in: *Ciba Foundation Symposium 143-The Biology of Hyaluronan: The Biology of Hyaluronan* (Wiley Online Library, 2007), pp. 233-247.

7. R. Stern, A. Asari, K. Sugahara. Hyaluronan fragments: an information-rich system. *Eur J Cell Biol* **85(8)**, 699–715 (2006).

8. K. Prydz. Determinants of glycosaminoglycan (GAG) structure. *Biomolecules* **5(3)**, 2003–2022 (2015).

9. S. Clark, T. Keenan, H. Fielder, L. Collinson, R. Holley, C. Merry, *et al*. Mapping the differential distribution of glycosaminoglycans in the adult human retina, choroid, and sclera. *Invest Ophthalmol Vis Sci* **52(9)**, 6511–6521 (2011).

10. H. Yuan, M. Tank, A. Alsofyani, N. Shah, N. Talati, J. LoBello, *et al*. Molecular mass dependence of hyaluronan detection by sandwich ELISA-like assay and membrane blotting using biotinylated hyaluronan binding protein. *Glycobiology* **23(11)**, 1270–1280 (2013).

© 2024 Chondrex, Inc. All Rights Reserved, 6049 1.0

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com