General Hyaluronic Acid (HA) ELISA Kit

Catalog No.: RK09299

version: 2.0

This package insert must be read in its entirety before using this product

Introduction

This ELISA kit used for quantitative determination of HA in serum, plasma, cell culture supernatant, and other biological fluids.

Principle of the Assay

This assay is based on the competitive binding technique in which HA present in a sample competes with a fixed amount of horseradish peroxidase (HRP)-labeled HA for sites on a monoclonal antibody. Following a wash to remove excess conjugate and unbound sample, a chromogenic substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped by acid, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of HA in the sample.

Material Provided & Storage Conditions

Unopened kits can be stored at 2-8°C for 1 year, and opened products must be used within 1 month.

Part	Size	Cat. No.	Storage of opened/reconstituted material
General HA Antibody Coated Plate	8 x12	RM43012	Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8℃ for 1 month.
General HA Standard Lyophilized	2 vials	RM43013	It is not recommended to use again after redissolving.
General HA Concentrated HRP- Conjugate Antigen(100x)	1 x60μL	RM43014	Store at 2-8℃ for 1 month *

Standard/Sample Diluent (R1)	1 x20 mL	RM00023	Store at 2-8℃ for 1 month *
HRP- Conjugate Antigen Diluent(R2)	1 x6 mL	RM00024	
Wash Buffer(25x)	1 x20 mL	RM00026	
TMB Substrate	1 x12 mL	RM00027	
Stop Solution	1 x6 mL	RM00028	
Plate Sealers	4 Strips		
Specification	1		

*Note: The specifications listed in the table are for 96T kit, and the amount of other components in the 48T kit are halved except for the standard, please be aware of this.

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

*For Research use only，not be used for diagnosis.

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. If the OD value of the sample obtained from the test exceeds the maximum detection limit of the product, please dilute the sample using the standard/sample diluent (R1) in the product. Therefore, it is recommended to pre-test the sample before formally testing the sample.
4. Sample addition, plate washing, incubation time, incubation temperature and other operations during the experiment will affect the final results, please strictly manage the experimental process and keep good records.
5. Variations in sample collection, processing, and storage may cause sample value differences.
6. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
7. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.

8. Stop Solution contains strong acid. Wear eye, hand, and face protection.
9. To ensure the best results, please refer to the labels or instructions for storage of relevant reagent components.
10. Mixing of the reagents after preparation is very important for the results, but some proteins or antibodies may be very sensitive to vigorous vortexing, which may cause loss of activity, so please use vortexing with caution.
11. Please use sterilised consumables for reagent preparation to avoid contamination of the reagents, which may affect the final test results.
12. In order to ensure the best detection effect, it is not recommended to reuse the working solution of the solubilised standard protein and related reagents after freezing.
13. The kit should be away from light when it is stored or incubated.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use

up within 1 months.

16. The 48T kit is also suitable for the specification.

Sample Collection & Storage

Cell Culture Supernatant: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 xg within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Other biological fluids : Centrifuge samples for 20 minutes at

1,000 xg. Collect the supernatants and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

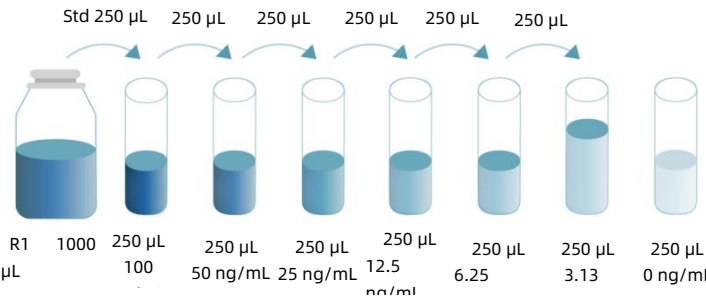
Note: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Standard - Reconstitute the Standard Lyophilized with 1.0 mL Standard/Sample Diluent(R1). This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use the 200 ng/mL. standard stock to produce a dilution series (below) with Standard/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 200, 100, 50, 25, 12.5, 6.25, 3.13, 0 ng/mL). Use diluted standards within 60 minutes of preparation.



Working HPR-Conjugate Antigen - Dilute 1:100 of Concentrated HRP-Conjugate Antigen (100x) with HRP-Conjugate Antigen Diluent (R2) before use. For example: Add 10 µL of Concentrated HRP-Conjugate Antigen (100x) to 990 µL HRP-Conjugate Antigen Diluent (R2) to prepare 1000 µL Working HRP-Conjugate Antigen Buffer.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:25 with double distilled or deionized water before use, for example : Add 16 mL of Wash Buffer Concentrate to 384 mL of deionized or distilled water to prepare 400 mL of Wash Buffer.

Sample preparation

For different samples, the appropriate dilution level should be chosen on a case-by-case basis.Please dilute the sample using the standard/Sample diluent(R1) in the kit.

1. Cell Supernatant: As cell supernatant samples vary considerably depending on the experimental conditions, it is recommended to carry out a pre-test to determine the appropriate dilution.For sample dilution, use standard/Sample diluent (R1) or PBS.

2. Serum/plasma:Due to individual differences, please anticipate the concentration range of the sample in advance and determine the dilution of the sample to be examined by pre-testing.Please refer to the following dilution instructions.For sample dilution, use standard/Sample diluent(R1) or PBS.

Dilution Method

For 100 fold dilution: One-step dilution. Add 5 μL sample to 495 μL sample diluent(R1) to yield 100 fold dilution.

For 1000 fold dilution: Two-step dilution. Add 5 μL sample to

95 μL sample diluent(R1)(1x) to yield 20 fold dilution, then add 5 μL 20 fold diluted sample to 245 μL sample diluent(R1)(1x), after this, the neat sample has been diluted at 1000 fold successfully.

Each dilution step should be performed at a minimum of 3 μL and at a maximum of 100-fold dilution. Each dilution step should be mixed well to avoid foaming.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Prepare the Concentrated HRP-Conjugate Antigen(100x) Working Solution 15minutes early before use.
3. Add 50 µL Standard/sample Diluent (R1) in a blank well. Add 50 µL different concentration of standard or sample in other wells. Add 50 µL Working HRP-Conjugate Antigen in each wells, and cover with new adhesive sealer provided.Cover with the adhesive strip provided. Incubate for 60 min at 37℃.
4. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
5. Remove residual liquid in the wells. Add wash buffer 350 µ L/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.

6. Add 100 µL TMB Substrate to each well. Incubate for 15-20 minutes at 37°C. Protect from light.
7. Add Stop Solution (50 µ L/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may cause higher value and less accurate result.

Assay Procedure Summary

Prepare the standard and reagents

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Add 50 µL of standards or test samples to each well

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Add 50 µL Working HRP-Conjugate Antigen

Incubate for 60min at 37°C, then wash 5 times

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Add 100µL Substrate Solution

Incubate for 15-20 min at 37°C under dark condition

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Add 50µL Stop Solution

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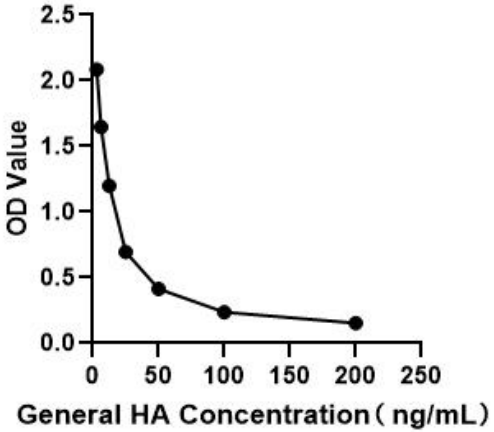
Detect the optical density within 5 minutes under 450 nm.

Correction Wavelength set at 570 nm or 630 nm

Calculation of Results

1. Calculate the average OD value of the replicate wells for each concentration of standard protein, quality control, sample, etc. The OD value of each test should be subtracted from the OD value of the sub-wavelength.
2. Create 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 a log/log graph. The data may be linearized by plotting the log of the EGF concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of HA assayed.

Detection Range

3.13-200 ng/mL

Sensitivity

The minimum detectable dose (MDD) of HA typically less than 1.56 ng/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural HA.
The method has high sensitivity and specificity for the detection of HA.
There is no significant cross-reactivity or interference between HA and analogues.

Note:
Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between HA and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-plate Precision

3 samples with low, middle and high level HA were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level HA were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	7.8	25	150	7.8	25	150
Standard deviation	0.25	1.55	5.55	0.5	2.23	10.65
CV(%)	3.2	6.2	3.7	6.4	8.9	7.1

Recovery

Matrices listed below were spiked with certain level of HA and the recovery rates were calculated by comparing the measured value to the expected amount of HA in samples.

Sample	Average Recovery（%）	Range（%）
Cell Culture Media(n=5)	90	87-98
Serum(n=5)	93	86-103

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of HA and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media(n=5)	Serum(n=5)
1:2	Average of Expected（%）	96	94
	Range（%）	85-113	85-103
1:4	Average of Expected（%）	90	100
	Range（%）	83-103	92-103
1:8	Average of Expected（%）	95	90
	Range（%）	90-104	88-97
1:16	Average of Expected（%）	93	98
	Range（%）	89-100	93-107

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.

	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

*For research purposes only. Not for therapeutic or diagnostic purposes.