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User's Manual

Hyaluronidase Activity ELISA Kit

REF **DEIA-XYZ14**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

96-well ELISA Assay for Detection and Quantification of Hyaluronidase Activity

General Description

Hyaluronidases are a group of enzymes that degrade Hyaluronan (HA), a linear polysaccharide comprised of a repeating disaccharide of N-acetylglucosamine and D-glucuronic acid. HA is involved in many biological processes including structural support, cell migration and tissue turnover. Circulating HA levels have also been shown to correlate in several disorders such as liver disease and certain types of cancer. Hyaluronidases have become an important area of study due to their regulatory function in HA metabolism and their role in physiological processes such as fertilization and wound healing. Research has also shown that hyaluronidases are involved in several pathological processes such as bacterial pathogenesis, the spreading of toxins/venoms and cancer progression, making hyaluronidases a potential pharmacological target.

Principles of Testing

CD Hyaluronidase Activity ELISA is a quantitative immunoassay designed for in vitro measurement of hyaluronidase activity in biological samples. Hyaluronidase reactions are performed in a 96-well microtiter plate pre-coated with HA substrate. The activity of the hyaluronidase is determined by comparing HA substrate levels post reaction to a standard curve of pre-coated HA substrate amounts. CD Hyaluronidase Activity ELISA has been validated with human sera, plasma and urine samples in addition to purified hyaluronidase from bovine testes. CD Hyaluronidase Activity ELISA provides a robust and simple method for researchers to measure hyaluronidase activity in biological samples.

Reagents And Materials Provided

1. Reaction Plate, 1 Plate
2. 5× Reaction Buffer, 1 Bottle
3. HA Detector, 1 Bottle
4. Secondary Detector, 1 Bottle
5. Hyaluronidase Control, 1 Vial
6. 10× PBS-T Buffer, 1 Bottle
7. Protein Stabilizer, 1 Vial
8. TMB Solution, 1 Bottle
9. 1N H_2SO_4 Stop Solution, 1 Bottle
10. Microtiter plate seal, 3 Seals

Materials Required But Not Supplied

1. Microtiter plate reader capable of reading absorbance at 450 nm
2. Incubator set at 37°C
3. Plate shaker or similar
4. Pipettes (20 µL, 200 µL, and 1,000 µL)
5. Reagent grade water

Storage

Kit: store at -20°C

Pack: store Part 1 at -20°C and Part 2 at 4°C to -20°C

Specimen Collection And Preparation

1. Samples should be free of any debris that may interfere with the assay.
2. A sample dilution of 1:20 is the minimum dilution recommended. Hyaluronidase activity can vary depending on the sample, so testing multiple dilutions may be beneficial.
3. When testing plasma, avoid using plasma prepared with heparin.
4. Sample buffers/diluents should be run as controls in the assay

Assay Procedure

Assay Notes:

1. The provided 5X Reaction Buffer must be used for the enzyme reactions. The 5× Reaction Buffer is optimized for hyaluronidases requiring acidic conditions. The pH of the reaction buffer should be adjusted if the samples require neutral conditions.
2. Do not let the Reaction Plate dry out once the assay has started. Always prepare the next solution needed prior to discarding the previous solution.
3. All reagents should be used immediately once they are diluted to working concentrations and cannot be saved for future use.

Procedure:

Please read this entire section, Assay Notes, and Sample Preparation sections before beginning.

1. Bring kit reagents to room temperature before use except the HA Detector, Secondary Detector and Hyaluronidase Control. Keep these reagents at -20°C until use.
2. Prepare 1× PBS-T buffer by diluting 30 mL of the 10× PBS-T Buffer with 270 mL dH_2O.
3. Prepare 1× Reaction Buffer by diluting 4 mL of the 5× Reaction Buffer with 16 mL dH_2O.
4. Prepare the Hyaluronidase Control by adding 1 mL of the 1× Reaction Buffer to the vial for a 1 U/mL concentration. Mix gently to fully resuspend the enzyme. The enzyme should be diluted just prior to use. Running the Hyaluronidase Control is an optional step but is recommended. A minimum of 1 duplicate data point should be run. Additional data points/dilutions can be run.
5. Prepare samples by diluting 1:20 with 1× Reaction Buffer (see"Sample Preparation" section). It is recommended that samples be run in duplicate according to the suggested plate layout. Samples should be

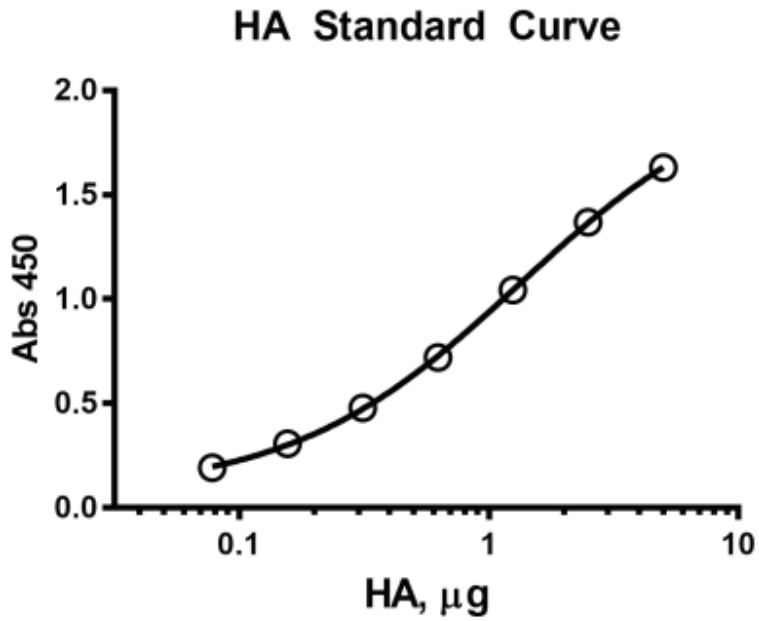
diluted just prior to use.

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µg	5 µg	HAase Control	HAase Control	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31
B	2.5 µg	2.5 µg	No HAase	No HAase	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
C	1.25 µg	1.25 µg	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
D	0.625 µg	0.625 µg	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
E	0.313 µg	0.313 µg	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
F	0.156 µg	0.156 µg	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
G	0.078 µg	0.078 µg	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
H	No HA	No HA	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38

6. Add 100 µL/well of the diluted samples and Hyaluronidase Control to the Reaction Plate according to the plate layout below. A "No Hyaluronidase" control should also be run using 100 µL/well of the 1× Reaction Buffer. Columns 1-2 of the Reaction Plate are pre-coated with the HA substrate standard curve. The remaining columns (3-12) are coated with 5 µg HA substrate/well that are to be used for samples and controls. No samples or controls should be added to the HA standard columns.
7. Cover the Reaction Plate with a plate seal. Incubate the reaction at 37°C. Incubation times may vary depending on the Hyaluronidase activity within samples. Suggested incubation time–30 minutes to 3 hours.
8. After incubation, prepare the HA Detector working solution by adding 12 mL 1× PBS-T buffer and 120 µL Protein Stabilizer to the bottle of HA Detector. Mix gently to fully resuspend the protein. Place prepared HA Detector at room temperature and proceed immediately to the next step. Do not leave prepared HA Detector at room temperature for more than 10 minutes.
9. Discard solution from plate and wash 4X with 200 µL/well 1× PBS-T buffer. Add 100 µL of the diluted HA Detector (step 8) to all wells of the Reaction Plate. Cover plate with a plate seal and incubate for 1 hour at room temperature on a plate shaker.
10. After the incubation, prepare the Secondary Detector working solution by adding 12 mL of PBS-T to the bottle of Secondary Detector. Mix gently to fully resuspend the Secondary Detector and set at room temperature until use. Do not leave prepared Secondary Detector at room temperature for more than 10 minutes.
11. Discard solution from plate and wash 3× with 200 µL/well 1× PBS-T buffer. Add 100 µL of the diluted Secondary Detector (step 10) to all wells of the Reaction Plate. Cover plate with a plate seal and incubate for 30 minutes at room temperature on a plate shaker.
12. After the incubation, wash plate three times with 200 µL 1× PBS-T buffer per well.
13. Add 100 µL of the TMB solution to each well of the Reaction Plate. Let blue color develop for 30 minutes in the dark.
14. Add 50 µL of the 1 N H_2SO_4 Stop Solution to each well to stop the reaction. Tap plate to mix.
15. Read absorbance at 450 nm.
16. Generate a best fit curve for the HA substrate standards in order to interpolate the remaining HA substrate in the reaction wells. Hyaluronidase activity can then be determined based on amount of HA substrate removed during the reaction time period.

Typical Standard Curve

HA standard curve was generated using non-linear regression analysis with GraphPad Software. A log[agonist] vs.response-variable slope (four parameter) analysis was utilized.



A sample titration using human serum with a 2 hour reaction time. Data interpolated against an HA standard curve and analyzed using non-linear regression analysis with GraphPad Software.