Mouse HS(Heparan Sulfate) ELISA Kit

Catalogue No.: IT6136

Size: 48T/96T

Reactivity: Mouse

Detection Range: 31.25-2000ng/ml

Sensitivity: <18.75ng/ml

Application: For quantitative detection of HS in serum, plasma, tissue homogenates and other biological fluids..

Storage: 4°C for 6 months

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(48T/96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×6 or 8×12	4°C/-20°C
Lyophilized Standard	1 vial or 2 vial	4°C/-20°C
Sample / Standard dilution buffer	10ml/20ml	4°C
Biotin- detection antibody (Concentrated)	30ul/60ul	4°C
Antibody dilution buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	4°C(shading light)
SABC dilution buffer	5ml/10ml	4°C
TMB substrate	5ml/10ml	4°C(shading light)
Stop solution	5ml/10ml	4°C
Wash buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with HS. During the reaction, HS in the sample or standard competes with a fixed amount of HS on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to HS. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution

and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of HS in the samples is then determined by comparing the O D of the samples to the standard curve.

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

Material Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automatic Washing

Aspirate all wells, and then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute.

Sample Collection and Storage

Isolate test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Place whole blood sample at room temperature for 2 hours or put it at 4°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- Tissue Homogenates: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Generally speaking, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5minutes at 5000×g to get the supernate.
- Cell Culture supernate: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernate and carry out the assay immediately.
- Other Biological Fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.
- Sample Preparation: Samples shall be clear and transparent and remove suspended solids by centrifugation.

Note: Samples to be used within 5 days can be stored at 4°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for this assay.

Sample Dilution Guideline

End user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. The following dilutions are for reference only:

- High concentration (20000-200000ng/ml): Dilution: 1:100. (i.e. Add 1µl of sample into 99µl of Sample/Standard Dilution Buffer.)

- Medium concentration (2000-20000ng/ml): Dilution: 1:10.(i.e. Add 10µl of sample into 90µl of Sample/Standard Dilution Buffer.)
- Low concentration (31.25-2000ng/ml): Dilution: 1:2. (i.e. Add 50µl of sample into 50µl of Sample/Standard Dilution Buffer.)
- Very low concentration (≤31.25ng/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

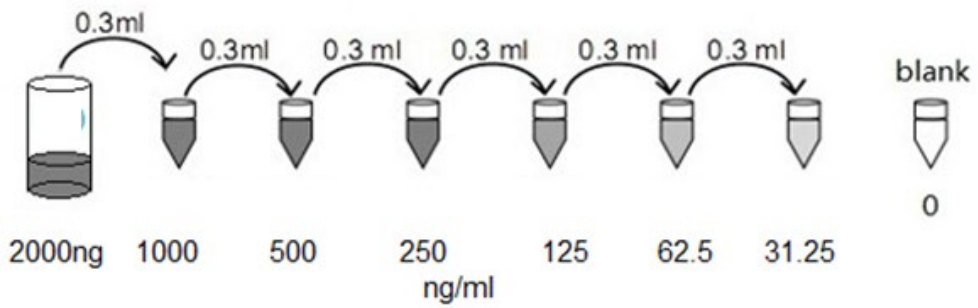
Put the kit at room temperature for 20 minutes before use.

1, Wash Buffer:

Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

1) 2000ng/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.
2) 1000ng/ml→31.25ng/ml of standard solutions: Label 6 Eppendorf tubes with 1000ng/ml, 500ng/ml, 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml , respectively. Aliquot 0.3 ml of the Sample/Standard dilution buffer into each tube. Add 0.3 ml of the above 2000ng/ml standard solution into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Biotin- labeled Antibody Working Solution

Prepare it within1 hour before experiment.

1) Calculate required total volume of the working solution: 0.05 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the Biotin- labeled Antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl of Biotin- labeled Antibody into 99µl of Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

1) Calculate required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1µl of SABC into 99µl of SABC dilution buffer.)

Assay Procedure

Before adding reagents into wells, equilibrate the TMB substrate for 30 min at 37 °C. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**

2. **Add Sample and Biotin- labeled Antibody:** Add 50µL of Standard, Blank, or Sample per well. The blank well is added with Sample/Standard dilution buffer. Immediately add 50µL Biotin-labeled Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
4. **HRP-Streptavidin Conjugate (SABC):** Add 100µL SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.
5. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.
6. **TMB Substrate:** Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 15-20 minutes.(The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
7. **Stop:** Add 50µL Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

8. **OD Measurement:** Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution.

Calculation of results

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD Value and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD Value of samples. Also, you can enter the corresponding fitting equation and OD Value of samples into Excel to get the concentration of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor. Recommended to use professional software curve expert to 1.3

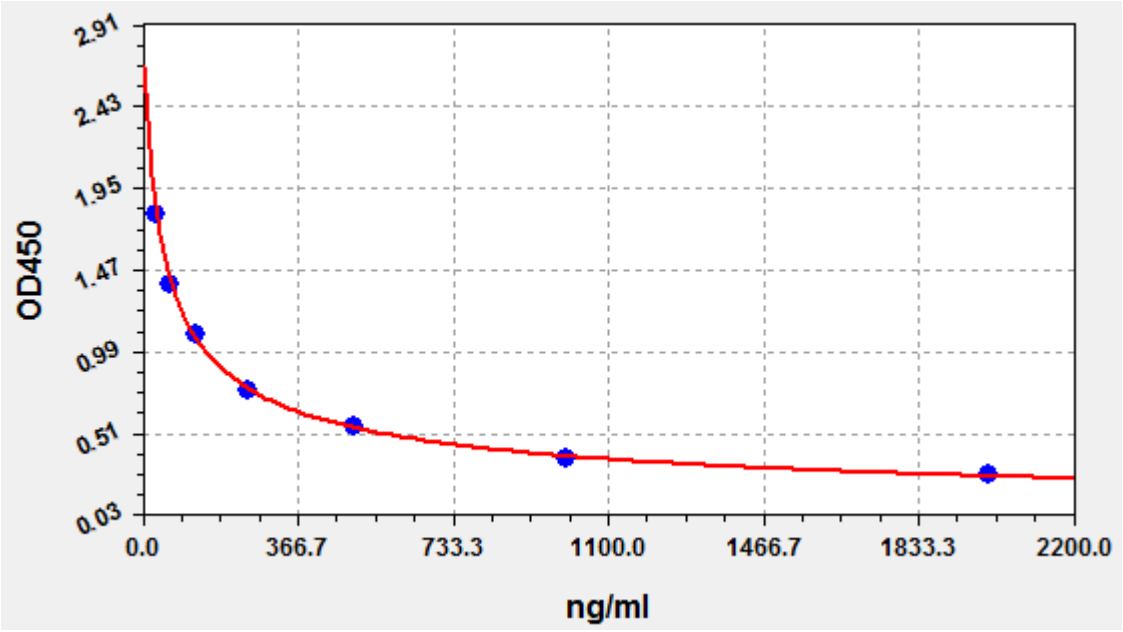
Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 50µL standard or sample into each well.
3. Immediately add 50µL Biotin- labeled antibody into each well.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash 3 times.
6. Add 100µL SABC Working Solution into each well. Incubate for 30 minutes at 37°C.
7. Aspirate and wash 5 times.
8. Add 90µL TMB Substrate. Incubate 15-20 minutes at 37°C.
9. Add 50µL Stop Solution. Read at 450nm immediately.
10. Calculation

Typical Data & Standard Curve

Results of a typical standard operation of a HS ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain standard curve as per experiment by themselves. (N/A=not applicable)

X	ng/ml	0	31.25	62.5	125	250	500	1000	2000
Y	OD450	2.674	1.797	1.389	1.093	0.765	0.555	0.362	0.272



Specificity

This assay has high sensitivity and excellent specificity for detection of HS . No significant cross-reactivity or interference between HS and analogues is observed.

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

Recovery

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

Matrix	Recovery range (%)	Average (%)
serum(n=5)	87-104	96
EDTA plasma(n=5)	88-104	98
heparin plasma(n=5)	85-102	91

Linearity

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

Sample	1:2	1:4	1:8	1:16
serum(n=5)	86-96%	85-94%	88-105%	85-99%
EDTA plasma(n=5)	82-96%	87-98%	87-90%	83-101%

heparin plasma(n=5)	88-99%	82-92%	83-97%	80-100%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level HS were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level HS were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by its activity rate. The activity of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	4°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.