Guinea Heparan Sulphate Proteoglycan Elisa Kit

96 Tests

Catalogue Number: E05H0130

Store all reagents at 2-8°C

Valid Period:six months

FOR LABORATORY RESEARCH USE ONLY.

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

This B.GHSPG ELISA kit is intended Laboratory for research use only and is not for use in diagnostic or therapeutic procedures.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration ofHSPG in the sample, thisHSPG ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versusHSPG concentration. The concentration ofHSPG in the samples is then determined by comparing the O.D. of the samples to the standard curve.

PRINCIPLE OF THE ASSAY

ThisHSPG enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific forHSPG. Standards or samples are then added to the microtiter plate wells andHSPG if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount ofHSPG present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific forHSPG are added to each well to “sandwich” theHSPG immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, A and B substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period.Only those wells that containHSPG and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1	MICROTITER PLATE	96 wells	
2	ENZYME CONJUGATE	10.0 mL	1 vial
3	STANDARD.1	0 pg/mL	1 vial
4	STANDARD.2	50pg/mL	1 vial
5	STANDARD.3	100pg/mL	1 vial

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6	STANDARD.4	250pg/mL	1 vial
7	STANDARD.5	500pg/mL	1 vial
8	STANDARD.6	1000pg/mL	1 vial
9	SUBSTRATE A	6.0 mL	1 vial
10	SUBSTRATE B	6.0 mL	1 vial
11	STOP SOLUTION	6.0 mL	1 vial
12	WASH SOLUTION x100	10 mL	1 vial
13	INSTRUCTION	1	
14.	LYSIS BUFFER SOLUTION	10.0ml	1 vial

NOTE: The LYSIS BUFFER SOLUTION is used only when the sample is cell culture fluid & body fluid & tissue homogenate ; If the sample is serum or blood plasma, then the LYSIS BUFFER SOLUTION is a superfluous reagent.

The kinds of sample:	
samplel:	serum or blood plasma
samplell:	cell culture fluid & body fluid & tissue homogenate

SAMPLE COLLECTION AND STORAGE

Serum-Use a serum separator tube(SST) and allow samples to clot for 30minutes before centrifugation for 15minutes at approximately 1000 x g.Remove serum and assay immediately or aliquot and store samples at -20 °C or -80°C.

Plasma-Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids-Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must stored at -20°C(≤2months) or -80°C(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles .When performing the assay slowly bring samples to room temperature.

DO NOT USE HEAT-TREATED SPECIMENS.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 ml to 1 ml volumes.
3. Adjustable 10ml -100ml pipettes for reagent preparation.
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5. 100 ml and 1 liter graduated cylinders.
6. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
7. Absorbent paper.
8. 37°C incubator.
9. Distilled or deionized water.
10. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired.
11. Tubes to prepare standard or sample dilutions.

1. BLUEGENE(BG) is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

6. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature (18-25 C) before use;

2. Dispense 10µl of LYSIS BUFFER SOLUTION into 100µl specimens, mix and stand for one hour (The proportion of LYSIS BUFFER and Specimens shall be no less than 1:10). (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate ; If the sample is serum or blood plasma, then this step should be skipped.)

3. Wash Solution - Dilute 10mL of Wash Solution concentrate (10×) with 990mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

ASSAY PROCEDURE

Prepare all Standards before starting assay procedure (**Please read Reagents Preparation)**. It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.

1. Secure the desired number of coated wells in the holder then add 50 µL of **Standards** or **Samples** to the appropriate well of the antibody pre-coated Microtiter Plate.

2. Add 100 µL of **Conjugate** to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37°C.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

4. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with wash solution, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of FIVE washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly hen washing the plate to assure that all strips remain securely in frame.

5. Automated Washing: Aspirate all wells, and then wash plate FIVE times using wash solution. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.

6. Add 50 µL Substrate A to each well.

7.Add 50 µL Substrate B to each well. **Cover** and incubate for 15 minutes at 20-25°C. (avoid sunlight)

8. Add 50 µL of Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

B.G CALCULATION OF RESULTS

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.

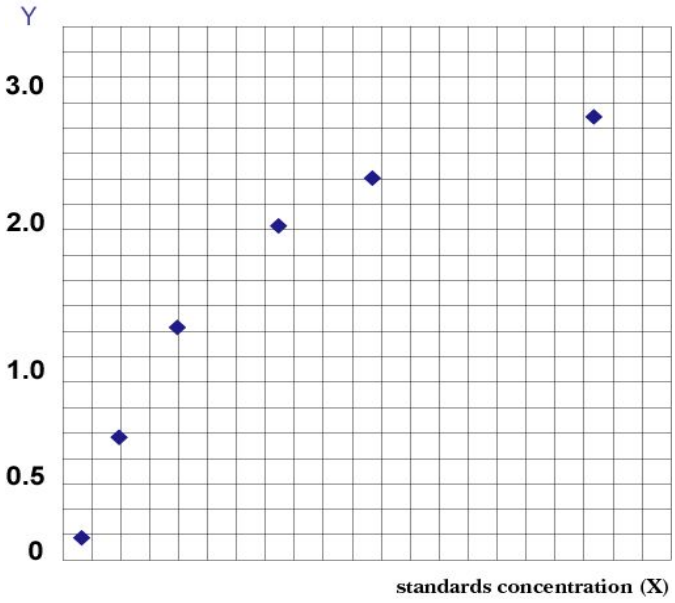
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.

3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

5. The sensitivity by this assay is 1.0pg/mL

6. Standard curve



Disposal Note Safety

1. This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for
disposal.

2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Additional Materials Required Quality Control

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.

2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.

4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.

6. It is recommended that all standards, controls and samples be run in duplicate.

7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

8. Cover or cap all reagents when not in use.

9. Do not mix or interchange different reagent lots from various kit lots.

10. Do not use reagents after the kit expiration date.

11. Read absorbances within 2 hours of assay completion.

12. The provided controls should be run with every assay. If control values fall outside pre-established ranges,the accuracy of the assay is suspect.

13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

14. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, or color may develop.

15. Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided.

16. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering anaspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

17. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under ASSAY METHOD. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

18. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbenttissue.

19. If using an automated washer, the operating instructions for washing equipment should be carefully followed.

20. Assay Procedure Preliminary notes: Do not mix reagents from different lots.It is recommended that assays be performed in duplicate.Standards and samples must be assayed at the same time.Avoid exposing the substrate to direct sunlight.

Note: this protocol is only for reference, mainly subject to the final package insert along with your order.