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Human GH ELISA Kit

Product Number: ELS-105262H

Components

Components	96 hole	48 hole
Microtiter plate	12*8strips	12*4strips
Standard(6 vial)	0.3ml/vial	0.3ml/vial
Dilution Buffer	6.0ml	3.0ml
Enzyme Conjugate	10.0ml	5.0ml
Buffer WS	25ml	15ml
Buffer A	6.0ml	3.0ml
Buffer B	6.0ml	3.0ml
Stop Buffer	6.0ml	3.0ml
Closure plate membrane	2	2

Note:1)Standard concentration was followed by: 16, 8, 4, 2, 1, 0.5 ng/mL.

2)If samples generate values higher than the highest standard, please dilute the samples with Dilution Buffer and repeat the assay.

3)Buffer WS (1X) - Dilute 1 volume of Buffer WS (20X) with 19 volumes of deionized or distilled water. Buffer WS is stable for 1 month at 2-8°C.

Description

This GH ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Buffer 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 of GH in the sample, this GH 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 versus GH concentration. The concentration of GH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Sample collection and storage

1. **Serum:** Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.
2. **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 ℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.
3. **Tissue homogenates:**For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is 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 centrifugated for 5minutes at 5000×g to get the supernate.
4. **Cell culture supernates and other biological fluids:**Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

   Note: The samples shoule be centrifugated dequately and no hemolysis or granule was allowed.

Materials required but not provided

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1. 37 ℃ incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper
4. Distilled or deionized water

Note

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
11. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
12. Buffer B contain 20% acetone, keep this reagent away from sources of heat or flame.
13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

Protocol

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.
2. Add 50μl of Standard or Sample to the appropriate wells. Blank well doesn't add anyting.
3. Add 100μl of Enzymeconjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
4. Wash the Microtiter Plate 4 times.
   - 4.1. 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 Buffer WS (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. 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 when washing the plate to assure that all strips remain securely in frame.
   - 4.2. Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350μL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
5. Add Buffer A 50μl and Buffer B 50μl to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
6. Add 50μl Stop Buffer to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

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 (X) axis versus the corresponding concentration on the horizontal (Y) 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 balnk well 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. Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.
6. Assay range: 0.5 ng/mL – 16 ng/mL.
7. Sensitivity: The minimum detectable dose of Human GH is typically less than 0.1 ng/mL.
8. Cross-reactivity: This assay recognizes recombinant and natural Human GH. No significant cross-reactivity or interference was observed.
9. Storage: 2-8°C (Use frequently); six months (-20°C)。
10. Standard curve.