RayBiotech
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Norcross, GA 30092, United States of America

Golgi Protein 73 (GP73) ELISA Assay Kit For in vitro diagnostics Catalog #: ELISA-GP73-96

• EXPECTED USAGE

This kit is designed for the quantitative detection of Golgi Protein 73 (GP73) in serum samples. This protein is often present at elevated levels in patients suffering from liver disease, notably in liver cancer patients.

• DIAGNOSTIC SIGNIFICANCE

Golgi Protein 73 (GP73), also known as golgi phospho protein 2 (GOLPH2), is a golgi type II transmembrane protein with a molecular mass of 73,000 Da. Recent studies have shown that levels of GP73 are abnormal in a variety of diseases. However, liver diseases show noticeable elevations in GP73 expression and presence in serum, especially in liver cancer. As such, this is a recently identified serum biomarker of liver cancer. Under normal circumstances, GP73 is an integral membrane protein on the membrane of the golgi apparatus. In a diseased state however, GP73 can dislodge from the golgi apparatus and reach endosomes to allow for exposure to the extracellular surface and spaces.

During hepatocellular carcinoma, diseased hepatocytes release excess GP73 into the serum. The level of GP73 can also be related to the stage of liver disease, with acute hepatitis showing higher levels, while chronic hepatitis C is characterized by lower levels. Finally, levels of GP73 can decline during various treatments possibly linking the efficacy of said treatment to the level of GP73. This kit functions to measure the level of extracellular release in a patient's serum sample using a sandwich-based ELISA method.

• DETECTION PRINCIPLES

Sandwich-Based ELISA Method:

Sandwich-based enzyme linked immunosorbent assay (ELISA) is based on a capture and detection antibody pair specific for the protein of interest. Initially, a specific anti-human GP73 monoclonal antibody is immobilized on the surface of a 96-well plate. Next, calibrators or samples are added into the wells of the plate. After incubation, a secondary detection antibody is added. Following secondary antibody incubation, an enzymatic conjugate is added that can bind antibody bound to the plate to create a complex of solid phase antibody-antigen-antibody. Upon addition of a coloring substrate, this enzyme reacts to produce a color relative to the amount of target GP73 present in the sample.

During the enzyme reaction, the color becomes blue, and changes to yellow in the presence of the stop solution. Finally, the OD of the wells at 450nm is read on a spectrophotometric plate reader. The OD450nm value is proportional to the concentration of GP73 in the sample.

• KIT COMPONENTS

The kit contains the following components. The entire kit can be used for 96 GP73 level measurements.

Components	Size
GP73 ELISA Plate	One 96 well plate
Plate Sealing Film	2 films
GP73 Enzyme Conjugate	One 10mL bottle
GP73 Calibration Vials	Five 0.5mL bottles (S1–S5)*
Wash Concentrate 20X	One 20mL bottle
Coloring Solution	One 1mL bottle
Stop Solution	One 7mL bottle
Instruction Manual	One copy

Special Note: The components of different lot numbers are not interchangeable

*The GP73 Calibration Vials S1-S6 contain 25ng/mL, 50ng/mL, 100ng/mL 200ng/mL, and 400ng/mL of GP73 recombinant antigen, respectively.

Additional Materials Required:

- Precision pipettes
- Distilled or deionized water
- 37°C or similar incubator
- 96-well microplate reader

• STORAGE AND STABILITY

Store kit at 2 – 8°C, away from moisture, and away from light. If stored properly, the kit is good for 12 months.

• SAMPLE COLLECTION AND STORAGE

Sample Requirements:

Only serum samples should be used. Samples that are hemolyzed, lipidemic, or jaundiced should be avoided.

Sample Isolation:

The kit is designed to test patient serum for the presence of GP73. Serum should be drawn into any appropriate tube and allowed to clot. Following clotting, the sample can be spun and the serum isolated off the clotted fraction.

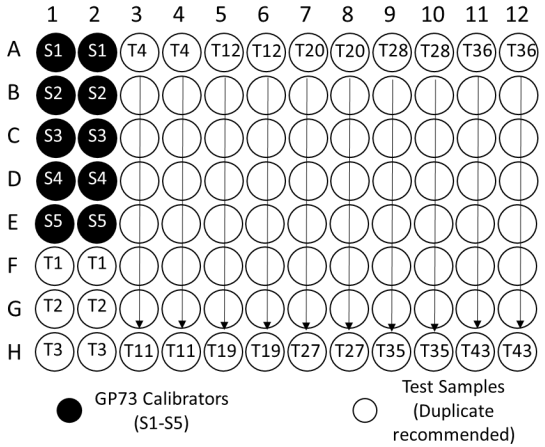
Sample Preservation:

Samples should be run immediately if possible. If not, store the collected serum sample at 0-4°C for 24 hours, or up to 3 months at -20°C. Avoid multiple freeze thaws.

• TESTING METHODS

1. Before testing, read the manual completely.
2. Ensure kit and sample have been stored correctly.
3. The reagents should be brought to room temperature before use. Avoid creating bubbles in reagents or sample dilutions that require mixing.
   - A standard curve should be run with each test.
   - If the sample concentration is too high, the sample should be diluted using the diluted 1x Wash Concentrate to bring it into linear range.
4. Prepare 1x Wash Concentrate by diluting the 20x Wash Concentrate 1:20 with distilled or deionized water (e.g. 20mL of 20x Wash Concentrate should be diluted with distilled water to a total volume of 400mL).
   - If crystallization occurs in the Wash Concentrate, it can be placed at 37°C to dissolve crystals before use.
   - Once reconstituted, 1x Wash Concentrate can be stored for up to 5 days at 4°C.
5. Number samples according to the corresponding microwells (example is shown below).

Example Plate Layout:



6. Add 20µL of the GP73 Calibrator or sample to the corresponding wells.
   - NOTE: it is recommended to run all samples and calibrators in duplicate.
7. Next, add 80µL of GP73 Enzyme Conjugate to each well and mix gently with rocking.
8. Seal the plate with the Plate Sealing Film and incubate for 60 minutes in a 37°C incubator.

- NOTE: Plate Sealing Films are one-time-use only and cannot be reused.

9. Remove the liquid from the wells and add 100µL of 1x Wash Concentrate to each well. Repeat 5 times.
10. Next, add 100µL of the Coloring Solution to each well and mix gently with rocking at 37°C in the dark for 10 minutes.
11. Add 50µL of Stop Solution to each well and tap gently to mix.
12. Evaluate the results with a microplate reader with the wavelength set to 450nm (recommended dual-wavelength 450/630nm detection).

• EVALUATION OF TEST RESULTS

Results Interpretation:

The plate should be read immediately following the addition of the Stop Solution. The concentration of GP73 in the sample is calculated from the generated calibration curve. The calibration curve should be generated by plotting the logarithm of the OD450 of each calibrator as the ordinate (Y-axis) and the logarithm of the concentration of each calibrator as the abscissa (X axis). Then, use a log-log linear fitting method determine the GP73 concentration.

Validity Evaluation:

The standard curve correlation coefficient (R^2) should not be less than 0.990 to meet the requirements of the test. The test is invalid should this value be <0.990.

Reference Range:

Our testing determined that the normal range of GP73 was less than 150ng/mL (320 non-disease patients tested).

- NOTE: The normal reference range is determined by a specific method. The clinical status and individual complexity differences, regional differences, and operator differences may affect the results of the test. It is recommended that each laboratory should establish its own reference range. These data are for reference only.

Results are determined as follows:

- **Negative result:** Quantity of GP73 indicated in the sample is less than or equal to150ng/mL
- **Positive result:** Quantity of GP73 indicated in the sample is greater than 150ng/mL

Limitations of the Assay:

- This kit is only used to assist in the diagnosis of liver disease against a reference patient cohort.
- These results are subject to the sample group evaluated, and results should be combined with other liver examination to confirm the results.
- Improper storage of the kit or it's reagents can compromise the kit results.
- Accurate measurement requires that the values not exceed the range of the calibration curve. Values that exceed this range should be diluted and run again.

Performance characteristics:

- Specificity: data and cross-reactivity with other substances are as follows:

Cross original	Concentration	Detected Concentration
Bilirubin	1000ng/mL	≤15ng/mL
Triglycerides	1000ng/mL	≤15ng/mL
Hemoglobin	1000ng/mL	≤15ng/mL
HE4	640 pmol/L	≤15ng/mL
CA125	200U/mL	≤15ng/mL
CA153	200U/mL	≤15ng/mL
AFP	1000ng/mL	≤15ng/mL
CEA	1000ng/mL	≤15ng/mL

- Blank values should not exceed 15ng/mL.
- Linearity: within the range of the calibration curve, the linear correlation coefficient (R^2) should be no less than 0.990.
- Accuracy: the recovery rate should be 85% to 115%.
- Repeatability: coefficient of variation CV ≤ 15%.
- Inter-assay Precision: coefficient of variation CV ≤ 15%.

• SAFETY MEASURES

Warning statements:

- This kit is intended for use only in accordance with the instructions contained herein and is intended for use as an in vitro diagnostic test kit within the specifications and limits described in the product description.
- This kit is limited to use in human serum samples for GP73 determination. Reliability in other body fluid samples has not yet been fully confirmed.
- Concentration values obtained by other methods and the results of this assay kit are not directly comparable.
- Patients who are in close contact with a rodent or who have received a monoclonal antibody preparation in a diagnostic treatment will have tropic antibodies in their samples, which may result in abnormal results.
- All work must conform to set biological safety code regulations to prevent cross-contamination.
- Specimens and enzyme-labeled reagents should be filled with a pipette that has been calibrated for accuracy.
- Operation of this kit should be strictly in accordance with the instructions. Different lots of reagents should not be mixed.
- The kit should be used for the quantitative determination of GP73 levels in samples. Test results must be combined with patient clinical information for analysis.
- Check the product expiration and packaging integrity before use. Do not use if the product is expired or packaging is damaged.
- All tests should be done in accordance with the relevant laboratory norms and requirements promulgated by the relevant state departments strictly to prevent cross-contamination.

Waste treatment:

- Following use, this product contains animal-derived substances that may be contaminated by the sample during operation. There are no known methods to fully ensure the presence of non-infectious substances. All reagent components, samples, and various wastes should be treated as infectious agents.

• REFERENCES

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4. Gu, Ann Clin Biochem. 2009 Jan;46(Pt 1):38-43.
5. Zinkin, Clin Cancer Res. 2008 Jan 15;14(2):470-7.

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