Version 25-02/25

User's Manual

# Human GPC3 ELISA Kit

REF DEIA3520V2

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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

This Kit is for the quantitative determination of human glypican-3 (GPC-3) concentrations in serum, urine, cell culture supernates.

## Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for GPC-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GPC-3 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for GPC-3 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GPC-3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## Reagents And Materials Provided

1. Assay plate (12 x 8 coated Microwells) 1(96 wells)
2. Standard (Freeze dried) 2
3. Biotin-antibody (100 x concentrate) 1 x 120 µl
4. HRP-avidin (100 x concentrate) 1 x 120 µl
5. Biotin-antibody Diluent 1 x 15 ml
6. HRP-avidin Diluent 1 x 15 ml
7. Sample Diluent 1 x 50 ml
8. Wash Buffer (25 x concentrate) 1 x 20 ml
9. TMB Substrate 1 x 10 ml
10. Stop Solution 1 x 10 ml
11. Adhesive Strip (For 96 wells) 4
12. Instruction manual 1

## Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 ml and 500 ml graduated cylinders.
6. Deionized or distilled water.

7. Pipettes and pipette tips.
8. Test tubes for dilution.

## Storage

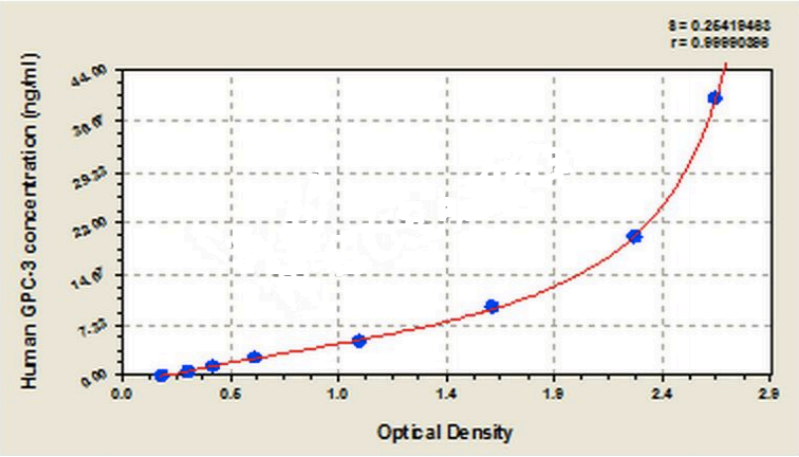
Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. Opened kit Coated assay plate May be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp. Standard/ Biotin-antibdy/ HRP-avidin May be stored for up to 1 month at 2 - 8°C. If don't make recent use, better keep it store at -20°C. Biotin-antibody Diluent/ HRP avidin Diluent/ Sample Diluent/ Wash Buffer/ TMB Substrate/ Stop Solution be stored for up to 1 month at 2 - 8°C.

## Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the GPC-3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Typical Standard Curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



| ng/ml | OD1 | OD2 | Average | Corrected |
|---|---|---|---|---|
| 40 | 2.621 | 2.641 | 2.631 | 2.437 |
| 20 | 2.356 | 2.194 | 2.275 | 2.081 |
| 10 | 1.626 | 1.674 | 1.650 | 1.456 |
| 5 | 1.021 | 1.101 | 1.061 | 0.867 |
| 2.5 | 0.596 | 0.600 | 0.598 | 0.404 |
| 1.25 | 0.412 | 0.422 | 0.417 | 0.223 |
| 0.625 | 0.302 | 0.315 | 0.309 | 0.115 |
| 0 | 0.187 | 0.201 | 0.194 | |

## Precision

Intra-assay Precision (Precision within an assay): CV%<8%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

## Detection Range

0.625 ng/ml-40 ng/ml

## Sensitivity

The minimum detectable dose of human GPC-3 is typically less than 0.156 ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

## Specificity

This assay has high sensitivity and excellent specificity for detection of human GPC-3. No significant cross-reactivity or interference between human GPC-3 and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human GPC-3 and all the analogues, therefore, cross reaction may still exist.

## Linearity

To assess the linearity of the assay, samples were spiked with high concentrations of human GPC-3 in various matrices and diluted with the Sample Diluent to produce samples with values within the dynamic range of the assay.

| | Sample | Serum(n=4) |
|---|---|---|
| 1:1 | Average % | 90 |
| | Range % | 80-100 |
| 1:2 | Average % | 98 |
| | Range % | 91-105 |
| 1:4 | Average % | 100 |
| | Range % | 92-110 |
| 1:8 | Average % | 93 |
| | Range % | 86-98 |

## Recovery

The recovery of human GPC-3 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Serum (n=5) | 95 | 89-105 |

## References

1. Values of circulating GPC-3 mRNA and alpha-fetoprotein in detecting patients with hepatocellular carcinoma, Yao M et al,Hepatobiliary Pancreat Dis Int.,2013 Apr;12(2):171-9.
2. Diagnostic value of glypican-3 in alpha fetoprotein negative hepatocellular carcinoma patients, Ozkan H et al,J Clin Lab Anal,2011;25(5):350-3.
3. Golgi protein 73, not Glypican-3, may be a tumor marker complementary to A-Fetoprotein for hepatocellular carcinoma diagnosis, Wang Y et al,J Gastroenterol Hepatol,2013 Nov 15.