GC-READ™ ITIH3 ELISA

For Research Use Only

INTENDED USE

GC-READ™ ITIH3 ELISA Test (**G**astric **C**arcinoma – **RE**combinant **A**ntigen-**A**ntibody **D**etection) is intended for qualitative and semi-quantitative detection of human Inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3) proteins in human plasma/serum. The test utilises sandwich ELISA using two layers of antibodies (i.e. capture and detection antibody) to determine a patient's ITIH3 proteins levels in plasma/serum samples which aid in the diagnosis of Gastric Cancer.

SUMMARY AND EXPLANATION OF TEST

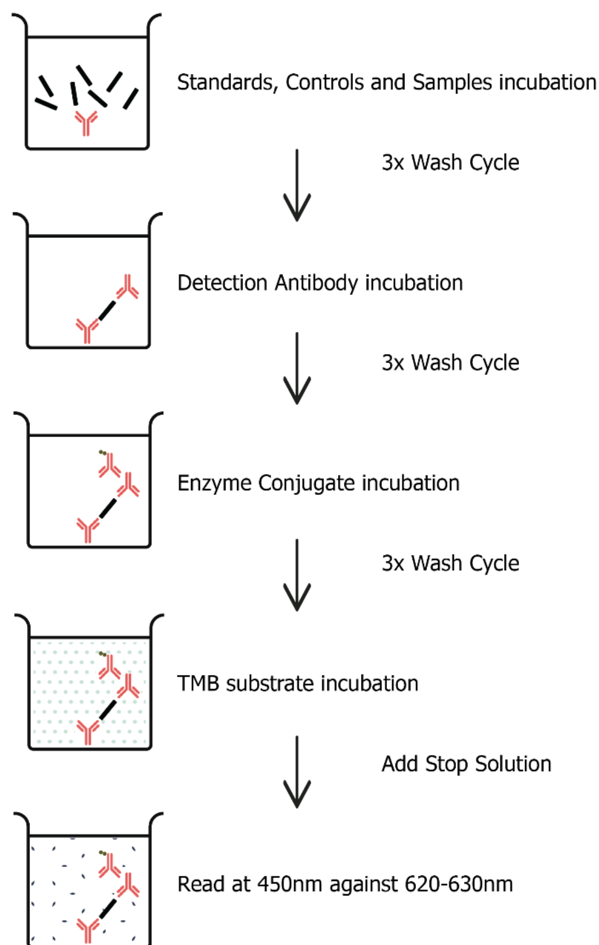
Gastric Carcinoma (GC) is the fifth most common cancer malignancy in the world with more than 70% of cases occur in developing countries, and half the world total occurs in Eastern Asia [3,5,6]. GC is the third leading cause of cancer death worldwide. The highest estimated mortality rates are in Eastern Asia. High mortality rates are also present in Central and Eastern Europe, and in Central and South America. The level of ITIH3 proteins has been found to be closely associated with Gastric Carcinoma. Higher levels of ITIH3 proteins were detected in plasma of patients with gastric carcinoma (both early and late) as compared to plasma of non-carcinoma patients [1,2,4].

GC-READ™ sandwich ELISA uses two layers of antibodies (i.e. capture and detection antibody) targeting at two different antigenic epitopes expressed by human ITIH3 proteins capable of binding to the respective antibody to semi-quantify the level of human ITIH3 protein. The test uses an arbitrary unit of measurement known as READ™-units (RU) which is used for determining the patients' GC risk level.

GC-READ™ is useful for the semi-quantification of human ITIH3 proteins in patients. All data from these assays, however at best offer supplementary information to clinicians and act as an aid to diagnosis only. It is therefore important that a diagnosis is made in conjunction with other recommended clinical investigations.

PRINCIPLE OF THE PROCEDURE

Human ITIH3 Capture Antibody is first coated onto the wells of microplates. Samples, standards and controls containing ITIH3 proteins are pipetted into these wells. During the first incubation, the protein antigen binds to the capture antibody and form antigen-antibody complexes. After washing, Human ITIH3 Detection Antibody is added to the wells and binds to the immobilised protein captured during the first incubation. After removal of excess detection antibody, a Horseradish Peroxidase (HRP) conjugate antibody is added and binds to the detection antibody. After a third incubation and washing to remove the excess HRP conjugate, a TMB substrate solution (tetramethylbenzidine) is added and is converted by the enzyme to a detectable form (color signal). The enzymatic reaction will then be stopped by the addition of 1N Sulphuric acid, which will turn the blue coloration to yellow. The microwells can be read on any suitable spectrophotometer or microwell ELISA plate reader. It is always recommended to read the wells at 450 nm against a 620-630 nm reference filter to eliminate any possible causes of interference. The intensity of this colored product is directly proportional to the concentration of antigen present in the original specimen.



MATERIALS PROVIDED

The amount of reagents is sufficient for 5 optimal runs.

Label	Reagent Constituents	Quantity
MICROPLATE	(Ready to use) 12 strips x 8 microwells coated with Human ITIH3 capture antibody	1 microplate
20X WASH BUFFER	(20X Concentrate) Phosphate buffer (~pH 7) with 1% Tween 20	50 mL
DILUENT	(Ready to use) Phosphate Buffer (~pH 7) with 0.05% Tween 20, 1% BSA	50 mL
STANDARD	(Ready to use) Human ITIH3 protein standard	2 mL
DETECTION ANTIBODY	(Ready to use) Human ITIH3 Detection antibody	15 mL
50X ENZYME CONJUGATE	(50X Concentrate) Secondary antibody coupled with horseradish peroxidase in stabilising buffer	300 µL
POSITIVE CONTROL	(Ready to use) Positive Control	1 mL
NEGATIVE CONTROL	(Ready to use) Negative Control	1 mL

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TMB SUBSTRATE SOLUTION	(Ready-to-use) Solution with 3,3',5,5' tetramethylbenzidine	15 mL
STOP SOLUTION	(Ready-to-use) 1N H_2SO_4 solution	15 mL
	Reseal-able bag for unused microwells	1

PRECAUTIONS

1. This kit is for research use only and should not be used as a diagnostic tool.
2. Some countries may regulate this test to be handled at Biosafety Level 2.
3. All components in the test kit have been quality controlled and tested against a Master Lot Unit. Pooling of any component is strictly not recommended; if there is sufficient balance to carry on testing, they should be use wholly on its own and never be pooled.
4. Reagents are only stable up till date of expiry and the manufacturer is not responsible for usage of expired reagents.
5. Assay set up must be carried out at room temperature. Any balance reagents that have been poured out should not be replaced into their original containers in case of cross contamination. All unused portions should be discarded appropriately.
6. Before opening the reagents, tap the vials firmly to ensure that the liquids are at the bottom (of the vials).
7. Do not use tap water. Strictly only deionised water can be use whenever required.
8. After each wash, ensure that reagents are added immediately to avoid wells drying up.
9. To avoid cross contamination of reagents, recap containers immediately and change gloves if there are any spills. This is one major reason for false results.
10. Over or under washing can result in assay variation and will affect validity of results.

ADDITIONAL MATERIALS REQUIRED

1. Measuring containers for wash buffer and diluents.
2. Timer (up to 30 minutes)
3. Pipettes capable of dispensing 10-200 µL and 200-1000uL with less than 3%CV.
4. Deionised or distilled water.
5. Paper towels.
6. Wash bottle, semi-automated or automated wash equipment.
7. Microplate spectrophotometer with dual wave length. Actual reading at 450 nm with reference of 620~630 nm.
8. Dilution tubes
9. Plate Shaker (approx. 350 rpm)

STORAGE AND STABILITY

1. All reagents must be stored at 2 ~ 8 ºC. Refer to the package for kit expiry.
2. Unopened microwells must be sealed in the reseal-able bag provided and stored at 2 ~ 8 ºC.

[NOTE: Only with proper and constant storage will kit be stable for the dating period specified.]

SPECIMEN COLLECTION

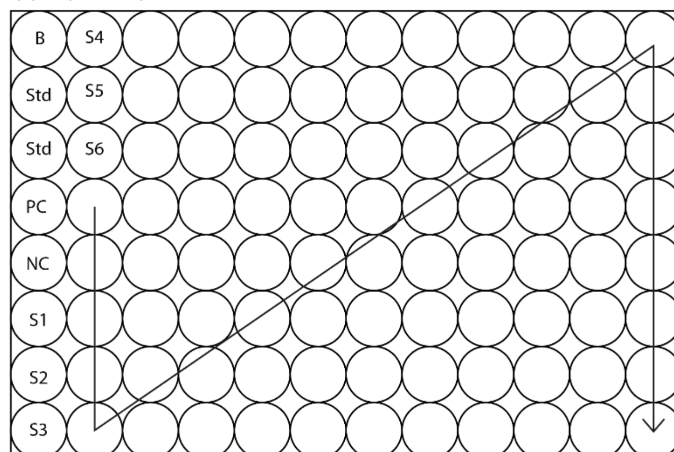
We would like to emphasize that the quality of the specimen is extremely important. Restalyst only warrants optimal performance if samples used in experiment are freshly collected that are clear, non-haemolysed, non-lipemic and non-icteric nature.

Recommended Collection & Storage:

1. All blood samples should be handling as if of infectious nature. Plasma/serum samples used should be freshly collected and prepared. Follow recommendations for order of draw outlined by WHO.[7]
2. All plasma / serum samples should be stored following recommendations set by WHO.[8]
3. Do not use plasma / serum samples older than one week. If not tested immediately, samples should be stored at 4°C.

PROCEDURE OF TEST

1. Ensure that all reagents are warmed up to room temperature prior to start. [Prepare 1X WASH BUFFER by diluting 50mL of 20X WASH BUFFER with 950mL distilled water]
2. Place the desired number of strips into the microwell frame. Recommendations: 1 well for blank, 2 wells for STANDARD, 1 well each for POSITIVE CONTROL & NEGATIVE CONTROL, and 1 well each for respective sample. *If using software for automated system, please check for configuration setup or contact software manufacturer for alternative recommended configuration. Additionally, ensure system is adequately maintained and calibrated appropriately.*



3. Dilute patient samples at 100-fold dilution using DILUENT (e.g. 10µL of sample + 990µL of DILUENT).
4. Pipette 100µL of DILUENT in blank well and 100µL of STANDARD, POSITIVE CONTROL, NEGATIVE CONTROL and diluted samples (prepared in step 3) into their respective well.
5. Incubate the microplate on the plate shaker (approx. 350 rpm) at room temperature for 30 minutes.
6. After incubation, wash the wells thoroughly either manually or by using a semi/fully automated washer. Use 300~350µL of 1X WASH BUFFER per well. A minimum of 3 wash cycles for manual wash are required while 5 cycles may be required for semi/fully automated washers.
7. Ensure that plate is tapped dry; add 100µL of DETECTION ANTIBODY to each well.
8. Repeat step 5.

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9. Meanwhile, prepare working Enzyme Conjugate by diluting 2 volumes of 50X ENZYME CONJUGATE with 98 volumes of DILUENT. (e.g. 2μL of 50x Enzyme Conjugate to 98μL of DILUENT).
10. Repeat step 6.
11. Ensure that plate is tapped dry; add 100μL of diluted Enzyme Conjugate to each well.
12. Repeat steps 5 and 6.
13. Ensure that plate is tapped dry; add 100μL of TMB SUBSTRATE SOLUTION to each well and incubate at room temperature in the dark for 15 minutes.
14. Then add 100μL of STOP SOLUTION to each well and mix well by tapping gently on the sides.
15. Place the microplate in the ELISA spectrophotometer/ reader to determine the optical density of each well, with primary filter at 450 nm and reference filter at 620~630 nm.

QUALITY CONTROL

For the assay to be valid:

1. Blank absorbance should be OD ≤ 0.200.
2. Standard absorbance should be 0.400 ≤ OD ≤ 0.700.
3. Positive Control absorbance should be OD ≥ 0.700.
4. Negative Control absorbance should be OD ≤ 0.300.

[NOTE: If the absorbance obtained is below the expected value, it signifies that the reagents have deteriorated]

CALCULATIONS

READ™-Units (RU) could be calculated as follow:

$$RU = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times 100$$

INTERPRETATION OF RESULTS

Calculate the RU value for each test sample as described above (section "CALCULATIONS")
Results are interpreted as follows:

RU of sample ≤72 = "Negative"
RU of sample >72 = "Positive"
With the cut-off of RU>72, the clinical specification of the assay is 95%.

METHOD VALIDATION

The performance evaluation is validated with 1025 Clinical Positive and Negative Samples. In ROC analysis, the true positive rate (Sensitivity) is plotted as a function of the false positive rate (100-Specificity) for different cut-off points. GC-REAAD™ was found to have a high clinical sensitivity of 91.0% and a high clinical specificity of 91.4%.

The test has also been found to not be affected by analytes such as haemoglobin, lipids (cholesterol & triglycerides), bilirubin and, proteins (total protein & blood albumin).

PRECISION

Intra-assay and inter-assay precision were determined by testing a positive control and a negative control in replicates of 12. GC-REAAD™ demonstrates an intra-assay %CV of 3.32% and 3.74% for positive and negative control respectively as well as an inter-assay %CV of 7.92% and 8.29% for positive and negative control respectively.

LIMITATIONS

1. This kit is for research use only and should not be used as a diagnostic tool.
2. Results from children should be viewed with caution.
3. Results obtained from immuno-compromised individuals should be interpreted with caution.
4. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
5. The performance characteristics have not been established for visual result determination.

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EFFECTIVE DATE: 02/01/2019

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