KAMIYA BIOMEDICAL COMPANY

Human Alpha GST ELISA, Serum

For the quantitative determination of human Alpha Glutathione S-Transferase in serum and sodium-heparinized plasma

Cat. No. KT-120

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Human Alpha GST ELISA, Serum is for the quantitative determination of Alpha Glutathione S-Transferase (αGST) in serum and sodium-heparinized plasma. It is intended for research use only. Not for use in diagnostic procedures.

DESCRIPTION
In liver, αGST is located in the hepatocytes whereas pi GST (πGST) is confined to the intrahepatic bile duct cells. This heterogeneous GST subclass distribution suggests that the isoenzymes have unique *in vivo* functions in different hepatic regions and that the detection of GST subclass levels in biological fluids would be of significant use in monitoring the integrity of specific hepatic regions.

The immunoassay of plasma or biliary πGST using Human Pi GST ELISA (Cat. No. KT-121) will provide further valuable complementary information regarding the biliary system. The parallel immunoassay of both α and πGST is thus particularly advantageous.

The **K-*ASSAY*®** Human Alpha GST ELISA, Serum is a specific, precise immunoassay for αGST and, being an ELISA, is unaffected by modulators of enzyme activity (e.g. bile salts and bilirubin).

PRINCIPLE
The **K-*ASSAY*®** Human Alpha GST ELISA, Serum is a quantitative enzyme immunoassay. The test procedure is based on the sequential addition of sample, antibody-enzyme conjugate and substrate to microtiter wells coated with anti-αGST IgG. The resultant color intensity is proportional to the amount of αGST present in the sample. The assay range is 0-40 µg/L.

COMPONENTS
- **Microtiter Plate:** 12x8-well strips of breakapart wells coated with anti-αGST IgG. READY TO USE.
- **αGST Calibrator Concentrate:** Purified αGST in stabilizing diluent (200 µL). Contains Thimerosal and sodium azide. STOCK SOLUTION.
- **Positive Control:** αGST in Protein containing solution with added stabilizers (4.5 mL). Contains Thimerosal and sodium azide. READY TO USE.
- **Conjugate Concentrate:** 51x anti-αGST IgG conjugated with horseradish peroxidase (HRP) (300 µL) Contains Thimerosal. CONCENTRATE.
- **Wash Solution Concentrate:** 20x Phosphate buffered saline/Tween-20 (PBST) (55 mL). Contains Thimerosal. CONCENTRATE.
- **TMB Substrate Solution:** Stabilized liquid TMB solution (11 mL). READY TO USE.
- **Stop Solution:** 0.5 M Sulfuric acid (11 mL). READY TO USE.
- **Plate Seal**
- **Package Insert**

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED
- Precision pipettes, 5-50, 50-200 and 200-1,000 µL and a multichannel pipette 50-200 µL
- Microplate washing system
- Microplate reader with 450 nm filter, with reference at 630 nm if available
- 1 L beaker
- Timer
- Liquid trough
- Deionized (distilled) water

- Plate shaker
- Graduated cylinder
- Test tubes

PRECAUTIONS

Safety

- For research use only. Not for use in diagnostic procedures.
- Intended for use by qualified laboratory staff only.
- This kit contains material of human origin which has been tested and found negative for hepatitis B virus DNA, hepatitis C virus RNA and HIV RNA. However, all recommended precautions for handling material of human origin should be observed.
- Some reagents contain Thimerosal, which may be toxic if ingested. The Stop Solution contains sulfuric acid, which is corrosive. Avoid contact with the skin and eyes. It contact occurs, rinse off immediately with water and seek medical advice.
- The Substrate contains TMB, which may irritate the skin and mucous membranes. Any substrate, which comes in contact with the skin, should be rinsed off with water.
- Some reagents contain sodium azide, which may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagents should be flushed with large volumes of water to prevent azide build up.
- Dispose of all specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed as though potentially infectious.
- Residues of chemicals, preparations and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not mouth-pipette the assay materials.
- Do not drink, eat or smoke in the area where assay materials are being handled.

Procedural

- Reagents with different lot numbers should not be mixed.
- Reagents should not be used after the expiration date specified on the kit label.
- Deviation from the protocol provided may cause erroneous results.
- 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.
- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry at any stage during the assay procedure.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution.
- Ensure Wash Solution Concentrate is mixed thoroughly and no crystals remain before reconstitution.
- High quality distilled or deionized water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background color in the assay.
- Allow all reagents to come to room temperature (20-25°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 4°C for extended periods.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Ensure that the upper surface of the wells is free of droplets before adding the next reagent. Drops should be gently blotted dry on completion of the washing steps.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay, an identification and distribution plan should be established.

PROTOCOLS

Preparation of Reagents

1. Wash Solution (PBST)
   Dilute Wash Solution Concentrate 1:20 by adding, for example, 10 mL Wash Solution Concentrate to 190 mL deionized water. Prepare only the volume of Wash Solution required for the assay.
   Ensure salt crystals are dissolved prior to dilution. Gentle warming of wash concentrate at 37°C for 30 minutes will aid dissolution of salt crystals.

2. Calibrators
   Prepare the 40 µg/L Calibrator (A) from the αGST Calibrator Stock Solution as follows:

   Stock Solution: 25 µL
   Wash Solution : 2,500 µL
   Total: 2,525 µL at 40 µg/L (Calibrator (A))

   Using labeled test tubes, prepare calibrators as follows:

To prepare Calibrator	Volume of Calibrator	Volume of Wash Solution
40 µg/L (A)	500 µL of Calibrator (A)	-----
20 µg/L (B)	500 µL of Calibrator (A)	500 µL
10 µg/L (C)	500 µL of Calibrator (B)	500 µL
5 µg/L (D)	500 µL of Calibrator (C)	500 µL
2.5 µg/L (E)	500 µL of Calibrator (D)	500 µL
1.25 µg/L (F)	500 µL of Calibrator (E)	500 µL
0 µg/L (G)	-----	500 µL

3. Conjugate
   Immediately prior to use, dilute the Conjugate Concentrate 1:51 by adding 20 µL Conjugate Concentrate to 1 mL Wash Solution per microtiter strip as follows:

No of Strips	Conjugate Volume (µL)	Wash Solution Volume (mL)
1	20	1
2	40	2
3	60	3
4	80	4
5	100	5
6	120	6
7	140	7
8	160	8
9	180	9
10	200	10
11	220	11
12	240	12

STORAGE

1. All kit reagents should be stored at 4°C and are stable as supplied until the expiration date.
2. αGST calibrators must be used within 30 minutes of preparation.
3. Working Wash Solution (PBST) is stable at RT for two weeks. Store at 4°C if extended storage is required.
4. Prolonged storage of diluted Conjugate at RT should be avoided. Use within 15 minutes of preparation.
5. Microtiter Plate break apart well strips should be stored in sealed bags with desiccant at 4°C until required for use. Return unused wells to the storage bag together with desiccant.

SAMPLE HANDLING AND STORAGE

Serum samples should be placed at -20°C for extended storage. No change in αGST levels has been observed in serum that has been stored at -20°C for up to 15 months. Repeated freeze-thawing of samples should be avoided in order to prevent loss of αGST. No significant differences have been observed between the recovery of αGST in sodium-heparinized plasma and serum.

SAMPLE PREPARATION

Immediately prior to the assay, dilute samples 1:5 by adding 50 µL sample to 200 µL Wash Solution. If multiple sample addition (>10 duplicate samples) is to be undertaken, then to facilitate transfer to the assay plate, samples may be diluted in a blank Microtiter Plate. The Positive Control does not require dilution.

FULL ASSAY PROCEDURE (1)

NOTE: All reagents should be allowed to reach RT prior to commencement of assay.

1. Sample / Calibrator incubation
   1.1 Prepare Wash Solution and Calibrators as described in "Preparation of Reagents".
   1.2 Prepare samples as described in "Sample Preparation".
   1.3 Place required number of microtiter wells in the assay plate (14 wells for Calibrators, and two each for the Control and samples). Arrange in columns of 8 wells and fill up spaces in the columns with blank microtiter wells. Add Calibrators (G-A; 0-40 µg/L), Positive Control and diluted samples (100 µL/well) in duplicate to the Microtiter Plate.
   1.4 Cover the Microtiter Plate and incubate at RT (20-25°C) for 60±2 minutes with uniform shaking.

2. Conjugate Incubation
   2.1 After 55 minutes prepare Conjugate as described in "Preparation of Reagents".
   2.2 Remove cover and wash each strip 4 times with Wash Solution (250–350 µL/well). When complete, firmly tap the plate against a paper towel to ensure complete removal of Wash Solution from the wells.
   Note: Either automated or manual washing is acceptable.
   2.3 Add 100 µL Conjugate/well.
   2.4 Again cover the Microtiter Plate and incubate at RT (20-25°C) for 30±2 minutes with uniform shaking.
   2.5 Wash each strip as in Step 2.2.

3. Color Development
   3.1 Add 100 µL Substrate/well using a multichannel pipette and incubate at RT in the dark for 15 minutes exactly.

4. Stop
   4.1 Add 100 µL Stop Solution/well using a multichannel pipette. Ensure complete mixing of Substrate and Stop Solution.
   4.2 Read immediately at 450 nm using 630 nm as reference (if available).

ANALYSIS OF RESULTS (1)

1. Calculate the mean absorbance for each Positive Control and sample.
2. Plot a calibration curve of $OD_{450\ nm}$ versus αGST concentration (µg/L). (See Figure 1.)
3. Read the concentration of αGST (µg/L) indicated by the mean absorbance of the samples from the calibration curve.
4. Multiply the calculated concentration of αGST by the appropriate dilution factor in order to obtain the actual αGST concentration.
5. The concentration of the Positive Control is read directly from the curve. Its value should be within the range given on the vial label.

MULTI-USE FORMAT

Once the calibration curve has been established using the full assay protocol, it is possible to perform subsequent sample analysis without preparing a calibration curve on each occasion (see Abbreviated Procedure (2) below) using the same plate and kit components. In order to ensure optimal interassay reproducibility the following instructions must be adhered to:

1. Subsequent assays must be run using the same plate and kit components as those used to generate the calibration curve.
2. Subsequent assays must be run within 21 days of generating the calibration curve. Otherwise a new calibration curve must be prepared.
3. Both **TEMPERATURE** and **INCUBATION TIMES** for abbreviated procedure must be identical with those used for calibration curve preparation (See "Full Assay Procedure" above). The use of an incubator for both the initial full procedure and subsequent assays is recommended.
4. The **K-*ASSAY*®** Human Alpha GST ELISA, Serum Positive Control should be included in all assays to allow the operator to monitor interassay precision.
5. It is recommended that all sample analyses are carried out in duplicate. The Positive Control should also be assayed in duplicate.
6. Once the assay is complete, the sample αGST concentration should be computed as described in "Calculation of Results (2)" below.

ABBREVIATED ASSAY PROCEDURE (2)

Note: 1) All reagents should be allowed to reach RT prior to commencement of the assay. 2) Both **TEMPERATURE** and **INCUBATION TIMES** for sample analysis must be identical with those used for the preparation of the calibration curve (For Full Assay Procedure, see above). **The use of an incubator is recommended.**

1. Sample /Calibrator Incubation
   1.1 Prepare Wash Solution as described in "Preparation of Reagents".
   1.2 Prepare Samples as described in "Sample Preparation".
   1.3 Place required number of Microtiter wells in the assay plate (two each for the controls and samples). Arrange in columns of 8 and fill up spaces in the columns with blank Microtiter wells. Add Positive Control and diluted samples (100 µL/well), in duplicate, to the Microtiter Plate.
   1.4 Cover the Microtiter Plate and incubate at RT (20-25°C) for 60±2 minutes with uniform shaking.

2. Conjugate Incubation
   2.1 After 55 minutes prepare Conjugate as described in "Preparation of Reagents".
   2.2 Remove cover and wash each strip 4 times with Wash Solution (250–350 µL/well). When complete, firmly tap the plate against a paper towel to ensure complete removal of wash fluid from the wells. Note: Either automated or manual washing is acceptable.
   2.3 Add 100 µL Conjugate/well.
   2.4 Again cover the Microtiter Plate and incubate at RT (20-25°C) for 30±2 minutes with uniform shaking.
   2.5 Wash each strip as in step 2.2.

3. Color Development
   3.1 Add 100 µL Substrate/well using a multichannel pipette and incubate at RT in the dark for 15 minutes exactly.

4. Stop
   4.1 Add 100 µL Stop Solution/well using a multichannel pipette. Ensure complete mixing of Substrate and Stop Solution.
   4.2 Read immediately at 450 nm using 630 nm as reference (if available).

ANALYSIS OF RESULTS (2)

1. Calculate mean absorbance for each sample and control.
2. Read the αGST concentration (µg/L), indicated by the mean absorbance of the sample, from the calibration curve previously generated using the same plate and kit components.
3. Multiply the calculated αGST concentration by the appropriate dilution factor in order to obtain the actual αGST concentration.
4. The concentration of the Positive Control is read directly from the curve.

QC CRITERIA

The positive control must always be included to assess the validity of the test results. Results are considered valid if the value of the Positive Control is within the range given on the Positive Control vial label.If this criterion is not met, the assay is considered invalid and must be repeated.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The sample detection limit of ***K-ASSAY®*** Human Alpha GST ELISA, Serum is 0.05 µg/L in the Microtiter well, 0.25µg/L in the sample.

Measuring Range

The calibration curve range covers 0-40 µg/L, corresponding to 0-200 µg/L in samples diluted 1:5 in Wash Solution. This range may be extended by increasing sample dilution.

Specificity

The ***K-ASSAY®*** Human Alpha GST ELISA, Serum is highly specific for the detection of αGST. No significant cross reactivity is observed with either mu or pi isoforms of GST as determined by EIA or immunoblot analysis.

Interference

No significant interference has been observed in this assay with lipemic, hemolytic or icteric sample.

Lipemic: Less than 10% interference up to 1,000 IU in sample.
Hemolytic: Less than 10% interference up to 1.17 g/L hemoglobin in the sample.
Iteric: Less than 11% interference up to 5 mg/mL bilirubin in the sample.

Some interference has been observed with plasma with plasma samples collected in EDTA and lithium heparin tubes.

In house studies have shown that samples with extremely high levels of rheumatoid factor may cause interference with this assay.

Precision

- Intra-assay (Within-Run) (n=20)

Sample	Mean αGST (µg/L)	SD	%CV
Low	0.79	0.12	15.3
Medium	56.05	3.6	6.42
High	154.72	20.26	13.09

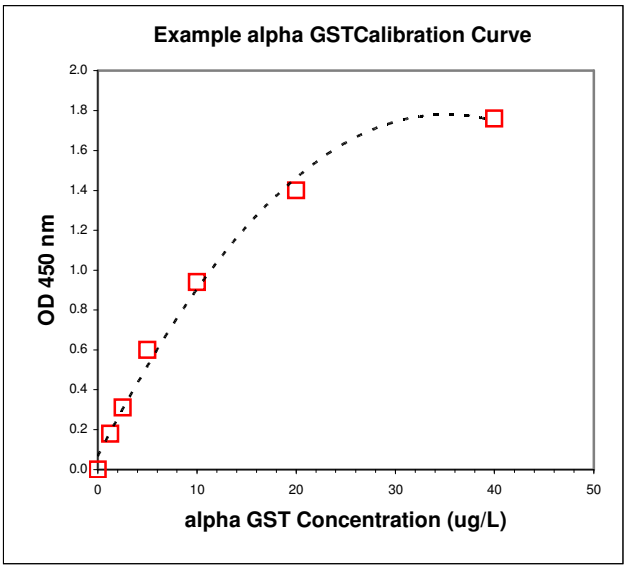
- Inter-assay (Run-to-Run) (n=10)

Sample	Mean αGST (µg/L)	SD	%CV
Low	1.03	0.25	24.41
Medium	51.54	5.81	11.27
High	135.41	34.07	25.16
Positive Control	12.84	1.12	8.68

- Inter-lot (Calculated across three lots of kits) (n=30)

Sample	Mean αGST (µg/L)	SD	%CV
Low	0.94	0.23	24.45
Medium	53.63	5.51	10.27
High	141.81	25.72	18.14

Figure 1. Typical Calibration Curve Using Human Alpha GST ELISA, Serum



Plot of A450 nm versus [αGST] µg/L. Assay range is 0-40 µg/L αGST.

Do not use this calibration curve for the calculation of results.

SUMMARY OF ASSAY PROCEDURE

1. Sample / Calibrator Incubation

1.1 Prepare Wash Solution and Calibrators.
1.2 Prepare samples.
1.3 Place microtiter wells in the assay plate. Add Calibrators, Positive Control and diluted samples **(100 µL/well)** in duplicate, to the Microtiter Plate.
1.4 Cover the Microtiter Plate and incubate at RT (20-25°C) **for 60±2 minutes** with uniform shaking.

2. Conjugate Incubation

2.1 After 55 minutes prepare conjugate as described in "Preparation of Reagents".
2.2 Remove cover and wash each strip 4 times with Wash Solution **(250-350 µL/well)**.
2.3 Add **100 µL** Conjugate/well.
2.4 Again, cover the Microtiter Plate and incubate at RT **for 30±2 minutes** with uniform shaking.
2.5 Wash each strip as in Step 2.2.

3. Color Development

3.1 Add **100 µL** Substrate/well and incubate at RT for 15 minutes exactly.

4. Stop

4.1 Stop the reaction by addition of **100 µL** Stop Solution/well. Ensure complete mixing of Substrate and Stop Solution.
4.2 Read immediately at 450 nm using 630 nm as reference (if available).

5. Calculate Results.

FOR RESEARCH USE ONLY

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