Alpha GST ELISA

For the quantitative determination of alpha glutathione S-transferase (αGST) in human urine, serum and plasma.

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 69-AGTHU-E01
Size: 96 Wells
INTENDED USE

The Alpha GST ELISA provides a method for the quantitative determination of alpha glutathione S-transferase (αGST) in human urine, serum and plasma. To assay αGST in other media or assay other GST subclasses, please contact ALPCO for further information.

BACKGROUND

URINE STUDIES

In the kidney’s, alpha glutathione S-transferase (αGST) is found in the proximal tubule region whereas Pi glutathione S-transferase (πGST) is confined mainly to the distal tubules. Low levels of αGST are released into the urine in normal individuals, as confirmed by immunoassay and Western blot analysis. Any event which precipitates proximal tubular damage may cause increased release of αGST into urine and elevations of urinary αGST levels have been shown to be indicative of proximal tubule damage in nephrotoxicity, environmental toxicity, surgery, acute renal failure and transplantation. The release of πGST has been shown to be associated with distal tubular damage, thus simultaneous measurement of αGST and πGST may allow discrimination between proximal and tubular damage.

SERUM STUDIES

In liver, alpha glutathione S-transferase 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. Currently, liver injury is studied by the measurement of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A disadvantage of these markers is that they are not distributed uniformly throughout the liver, the periportal concentration being greater than the centrilobular. In contrast, αGST has been found to be equally distributed in both the centrilobular and periportal regions. Since the centrilobular hepatocytes are very susceptible to damage in a variety of conditions including Allograft Rejection, Viral Hepatitis, and Hepatotoxicity, αGST is a more sensitive indicator of hepatic status.

The Alpha GST ELISA is a specific, precise immunoassay for αGST and, being a quantitative test, is unaffected by modulators of enzyme activity (e.g. bile salts and bilirubin). Thus, it is now possible to use αGST quantitation to study the hepatocellular status of individuals at risk of hepatic damage.

ASSAY PRINCIPLE

The Alpha GST ELISA is a quantitative enzyme immunoassay. The test procedure is based on the sequential addition of sample, antibody-enzyme conjugate and substrate to microassay 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 2.5 – 80 μg/L.
**COMPONENTS**

1. Antibody Coated Microassay Plate
   96 well (12x8 breakapart well strips coated with IgG directed against αGST)
   READY TO USE

2. Calibrator, 0.2mL (2mg/L)
   Purified αGST in stabilising diluent containing ProClin 950 and Bronidox L as preservatives.
   25X CONCENTRATE

3. Sample Diluent, 30mL
   Protein containing solution with added stabilizers and ProClin 950 and Bronidox L as preservatives.
   READY TO USE

4. Wash Buffer, 45mL
   Tris-buffered saline/Tween-20 (TBST) containing ProClin 950 as preservative.
   25X CONCENTRATE

5. Positive Control, 1.5mL
   Purified αGST in stabilising diluent containing ProClin 950 and Bronidox L as preservatives.
   READY TO USE

6. Enzyme Conjugate, 11mL
   Antibody solution containing anti-αGST IgG labelled with horseradish peroxidase and ProClin 950 and Bronidox L as preservatives.
   READY TO USE

7. Substrate, 11mL
   Stabilised liquid TMB solution
   READY TO USE

8. Stop Solution, 11mL
   0.5M Sulphuric Acid
   READY TO USE

9. Urine Stabilising Buffer (USB), 10mL
   Protein containing solution with added stabilizers and ProClin 950 and Bronidox L as preservatives.
   READY TO USE

10. Instructions for use
PRECAUTIONS

SAFETY

• Alpha GST ELISA is for research use only and not for use in diagnostic procedures.
• Alpha GST ELISA is intended for use by qualified laboratory staff only.
• The Stop Solution contains sulphuric acid, which is corrosive. Avoid contact with the skin and eyes. If 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.
• Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of 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 pipette materials by the mouth and never eat or drink at the laboratory workbench.
• The components containing ProClin 950 are classified as per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:
  R43 May cause sensitization by skin contact.
  S24 Avoid contact with skin.
  S35 This material and its container must be disposed of in a safe way.
  S37 Wear suitable gloves.
  S46 If swallowed, seek medical advice immediately and show this container or label.

PROCEDURAL

• Do not use kit or individual reagents beyond their expiration date.
• Do not mix or substitute reagents from different kit lot numbers.
• 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.
• High quality distilled or deionised water is required for the Wash Buffer. 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 2-8°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 wash step.
• 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.
STABILITY AND STORAGE

1. All kit reagents should be stored at 2-8°C and are stable as supplied until the expiry date shown.
2. Microassay wells should be stored in the sealed foil pouch with desiccants at 2-8°C until required for use. Return unused wells to the storage pouch together with desiccants.
3. Alpha GST Calibrators must be used within 30 minutes of preparation.
4. Prepared Wash Buffer (TBST) is stable at room temperature for two weeks or at 2-8°C for one month.

ADDITIONAL MATERIALS REQUIRED

1. Micropipettes and a multichannel pipette
2. Microassay strip washing system
3. ELISA plate reader capable of measuring at 450nm with reference at 630nm if available
4. Timer
5. Liquid trough
6. Graduated cylinder
7. Test tubes
8. Deionised/distilled water
9. Plate shaker
10. Room temperature incubator
11. Vortex

PREPARATION OF REAGENTS

1. **WASH BUFFER (TBST)**
   - Perform a 1/25 dilution of Wash Concentrate by adding, for example, 20mL 25X Wash Concentrate to 480mL deionised water as required. Prepare only the volume of Wash Buffer required for the assay. Each strip of 8 wells requires 25mL Wash Buffer.

2. **CALIBRATORS**
   - Prepare Calibrator (A) from the αGST stock solution as follows:

<table>
<thead>
<tr>
<th>αGST Calibrator Concentration (µg/L)</th>
<th>Calibrator Volume (µL)</th>
<th>Sample Diluent Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 (A)</td>
<td>300 (A)</td>
<td>0</td>
</tr>
<tr>
<td>40 (B)</td>
<td>300 (A)</td>
<td>300</td>
</tr>
<tr>
<td>20 (C)</td>
<td>300 (B)</td>
<td>300</td>
</tr>
<tr>
<td>10 (D)</td>
<td>300 (C)</td>
<td>300</td>
</tr>
<tr>
<td>5 (E)</td>
<td>300 (D)</td>
<td>300</td>
</tr>
<tr>
<td>2.5 (F)</td>
<td>300 (E)</td>
<td>300</td>
</tr>
<tr>
<td>0 (G)</td>
<td>0</td>
<td>300</td>
</tr>
</tbody>
</table>

Mix Calibrator (A) by vortexing for 5 - 10 seconds. Using labelled tubes prepare further calibrators as follows:
SAMPLE COLLECTION

URINE
The Alpha GST ELISA can be used to measure αGST in any urine sample but, due to the diurnal variation in proteinuria, it is important for optimal results that timed, quantitative, urine samples are collected and the collection period and volume recorded. This will enable αGST excretion to be expressed as rate (ng/min), refer to Appendix 1. Overnight or 24 hour urine samples are recommended. For the use of other collection methods and periods, contact ALPCO for advice.

As soon as possible after sample collection, add 100μL of Urine Stabilising Buffer to 400μL urine (4/5 dilution of sample), even if the samples are not to be stored. The presence of blood will not affect αGST measurements.

SERUM / PLASMA
The Alpha GST ELISA can be used to measure αGST in serum, EDTA or sodium-heparin plasma samples.

Collect all blood samples in an appropriate tube and observe routine precautions for venipuncture. Mix the tube immediately after collection by inverting several times. Centrifuge within 3 hours from time of collection and transfer the sample from the original tube for storage at 2-8°C. If not tested within 24 hours, aliquot the sample and store at -20°C or -80°C. Inspect samples for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

SAMPLE HANDLING AND STORAGE

URINE
Do not store urine samples without the addition of Urine Stabilising Buffer (USB). USB must be added within 12 hours of sample collection. It is recommended that samples are assayed as soon as possible after collection. However, after the addition of USB, samples can be stored at 20-25°C for up to 48 hours, at 2-8°C for up to one week or at -20°C for >1 year. Repeated freeze thawing of samples should be avoided to prevent loss of αGST (up to 20% drop in αGST concentration observed after 3 freeze-thaw cycles as measured by ELISA).

SERUM / PLASMA
Serum and plasma samples can be stored at 20-25°C for up to 48 hours, at 2-8°C for up to one week or at -20°C for >1 year. Repeated freeze thawing of samples should be avoided to prevent loss of αGST (up to 20% drop in αGST concentration observed after 3 freeze-thaw cycles as measured by ELISA).

SAMPLE PREPARATION

URINE
Immediately prior to the assay, dilute samples 1/2 by adding 125μL stabilised urine sample to 125μL Sample Diluent.

SERUM / PLASMA
Immediately prior to the assay, dilute samples dilute 1/5 by adding 50μL sample to 200μL Sample Diluent.
NOTE: If multiple sample additions (>10 duplicate samples) are to be undertaken then, to facilitate transfer to the assay plate, samples can be diluted in a blank microassay plate.

POSITIVE CONTROL
The positive control sample does not require dilution.
ASSAY PROCEDURE

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

1. SAMPLE / CALIBRATOR INCUBATION

   1.1. Prepare Wash Buffer and Calibrators as described in ‘Preparation of Reagents’.

   1.2. Prepare Samples as described in ‘Sample Preparation’.

   1.3. Place required number of microassay wells in the assay plate (14 for the Calibrators plus two for each of the controls and samples). Add Calibrators (G-A; equivalent concentration 0 - 80μg/L), Positive Control and diluted samples (100μL/well) in duplicate, to the microassay plate.

   1.4. Cover the microassay plate and incubate at room temperature (20-25°C) for 60 ± 2 minutes with uniform shaking (350 ± 10rpm).

   1.5. Remove cover and wash each strip 4 times with Wash Buffer (250μL - 350μL/well). When complete, firmly tap the plate against a paper towel to ensure complete removal of Wash Buffer from wells. Note: Either automated or manual washing is acceptable.

2. CONJUGATE INCUBATION

   2.1. Add 100μL Conjugate/well to the microassay plate using a multichannel pipette.

   2.2. Again cover the microassay plate and incubate at room temperature (20-25°C) for 30 ± 2 minutes with uniform shaking (350 ± 10rpm).

   2.3. Wash each strip as in Step 1.5.

3. COLOR DEVELOPMENT

   3.1. Add 100μL Substrate/well using a multichannel pipette and incubate at room temperature in the dark for 15 minutes exactly with NO shaking.

4. STOP

   4.1. Stop the reaction by adding 100μL Stop Solution/well using a multichannel pipette. Ensure complete mixing of Substrate and Stop Solution.

   4.2. Read immediately at 450nm using 630nm as reference (if available).
CALCULATION OF RESULTS

1. Calculate the mean absorbance for each sample.
2. Plot a calibration curve of $A_{450/630nm}$ versus $[\alpha GST]$ (μg/L) (4-parameter plot, refer to Figure 1).
3. Read the $[\alpha GST]$ (μg/L) indicated by the mean absorbances of the samples from the calibration curve.
4. Multiply the calculated $[\alpha GST]$ by the appropriate dilution factor in order to obtain the actual $[\alpha GST]$. Results for stabilised urine samples should be multiplied by an additional factor of 1.25 to compensate for the dilution of sample with Urine Stabilising Buffer.
5. The concentration for the Positive Control is read directly from the curve.
6. Concentrations of samples with readings outside the standard curve are invalid and must be repeated with a higher dilution factor. It is not acceptable to extrapolate data.

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 specified on the inside of the box lid. If the control is out of the specified range, the associated test results are invalid and must be re-tested.

PERFORMANCE CHARACTERISTICS

MEASURING RANGE
The calibration curve range is 2.5 - 80μg/L, which corresponds to 6.25 - 200μg/L in stabilised urine samples diluted 1/2 in Sample Diluent or 12.5 - 400μg/L in serum/plasma samples diluted 1/5 in Sample Diluent. This range may be extended by increasing sample dilution.

PRECISION
A 10-day precision study was performed on the Alpha GST ELISA based on guidance from the Clinical and Laboratory Standards Institute (CLSI) Document EP15-A2. Testing was performed on site using two lots of Alpha GST ELISA and 4 different operators. Three urine pools containing endogenous αGST and four control samples spiked with αGST were assayed in duplicate at two separate times per day for 10 days. The data is summarized in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean $[\alpha GST]$ (μg/L)</th>
<th>Repeatability</th>
<th>Between-Run</th>
<th>Between-Day</th>
<th>Within-Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Urine Pool</td>
<td>40</td>
<td>24.0</td>
<td>0.5%</td>
<td>2.2%</td>
<td>8.5%</td>
<td>3.6%</td>
</tr>
<tr>
<td>Medium Urine Pool</td>
<td>40</td>
<td>43.8</td>
<td>1.1%</td>
<td>2.5%</td>
<td>5.7%</td>
<td>2.8%</td>
</tr>
<tr>
<td>High Urine Pool</td>
<td>40</td>
<td>195.4</td>
<td>5.0%</td>
<td>2.6%</td>
<td>6.1%</td>
<td>6.5%</td>
</tr>
<tr>
<td>High Plasma</td>
<td>40</td>
<td>1581.6</td>
<td>86.7%</td>
<td>5.4%</td>
<td>89.91%</td>
<td>14.7%</td>
</tr>
<tr>
<td>Low Urine Control</td>
<td>40</td>
<td>15.3</td>
<td>0.3%</td>
<td>2.4%</td>
<td>6.6%</td>
<td>0.00%</td>
</tr>
<tr>
<td>Medium Urine</td>
<td>40</td>
<td>41.5</td>
<td>0.4%</td>
<td>1.2%</td>
<td>5.9%</td>
<td>0.5%</td>
</tr>
<tr>
<td>High Urine</td>
<td>40</td>
<td>75.0</td>
<td>1.7%</td>
<td>2.3%</td>
<td>5.8%</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
SPECIFICITY
Alpha GST ELISA is highly specific for αGST. No cross-reactivity was observed with μGST at 500µg/L, or πGST at 500µg/L.

SENSITIVITY
The limit of detection (LoD) of Alpha GST ELISA was estimated from 60 blank sample measurements and 60 replicates of low-level sample measurements as per CLSI Document EP17-A. The limit of detection was found to be 1.9µg/L αGST, which corresponds to 4.75µg/L in a stabilised urine sample diluted 1/2 or 9.5µg/L in a serum/plasma sample diluted 1/5.

LINEARITY UPON DILUTION
Sample pools with αGST concentrations ranging from 14.7µg/L to 15400µg/L were serially diluted with Alpha GST ELISA Sample Diluent and assayed. % Recovery of αGST recovery was calculated as (Measured [αGST] µg/L / Expected [αGST] µg/L) x 100. Recovery of αGST was found to be 100±10% (serum 92-105%, EDTA plasma 94-105%, heparin plasma 94-105%, and stabilised urine 91-107%).

INTERFERENCE
Potentially interfering endogenous substances were evaluated to determine their effect on αGST recovery using ALPCO Alpha GST ELISA. The endogenous substances listed below were spiked into urine and serum pools containing endogenous αGST at a concentration of ~300µg/L and assayed to determine the degree of interference. The degree of interference with each test substance is presented in the table below. The percentage bias for each interferent was calculated as:

\[
\text{% Bias} = \left( \frac{[\text{αGST}] \text{ µg/L interferent-spiked urine}}{[\text{αGST}] \text{ µg/L non-spiked urine}} \right) \times 100 - 100
\]

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>Interferent Concentration (mg/dL)</th>
<th>Interference in Urine (% Bias)</th>
<th>Interference in Serum (% Bias)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (conjugated)</td>
<td>20</td>
<td>0%</td>
<td>-4%</td>
</tr>
<tr>
<td>Bilirubin (unconjugated)</td>
<td>20</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>2000</td>
<td>-7%</td>
<td>-1%</td>
</tr>
<tr>
<td>Albumin</td>
<td>6000</td>
<td>3%</td>
<td>1%</td>
</tr>
<tr>
<td>Lipid*</td>
<td>1500</td>
<td>-5%</td>
<td>1%</td>
</tr>
<tr>
<td>Human IgG</td>
<td>4</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Tamm-Horsfall Protein**</td>
<td>5</td>
<td>-22%</td>
<td>-</td>
</tr>
</tbody>
</table>

* Performed with 20% intralipid.
** The endogenous concentration of the urine sample pool used for testing was unknown. The average THP concentration in healthy subjects is estimated at 6.1 – 9.0 mg/dL and thus, the final concentration is likely to be in excess of 11.1mg/dL.
No significant interference was observed in this assay with EDTA up to 3.4µmol/L or sodium heparin up to 3,000U/L. Studies also indicated that samples with rheumatoid factor do not cause interference.

**EXAMPLE OF A CALIBRATION CURVE**

![Chart](image)

**Figure 1.** Typical calibration curve obtained using ALPCO Alpha GST ELISA. 4-parameter plot of $A_{450/630nm}$ versus [αGST] μg/L. Assay range is 2.5 – 80 μg/L αGST.

**APPENDIX 1**

**EXPRESSING αGST RELEASE RATE**

Excretion of αGST is constant with time, not urine volume. This means that it may be more relevant to express αGST release in terms of rate (ng/min) rather than concentration. This can be important in situations of unusual diuresis, such as oligo or polyuria. The rate of release is obtained as follows:

**URINE COLLECTION**

Collect urine samples as described in ‘Sample Collection’. Note the time of urination (T2), time of the previous urination (T1) and the total urine volume (V).

**CALCULATION OF αGST EXCRETION RATE**

1. Determine urinary αGST levels (μg/L) using ALPCO Alpha GST ELISA.
2. Calculate the period over which the urine was collected ($T = T2 - T1$) in minutes.
3. Note the urine volume in mL (V).
4. Calculate the rate of release as follows:

$$\text{ng αGST/min} = \frac{[\alpha\text{GST}] \mu\text{g/L} \times V}{T}$$
SUMMARY OF ASSAY PROCEDURE

1. **SAMPLE/CALIBRATOR INCUBATION**
   1.1. Prepare Wash Buffer and Calibrators.
   1.2. Prepare Samples
   1.3. Place microtitre wells in the assay plate. Add Calibrators, Positive Control and diluted samples (100μL/well), in duplicate, to the microtitre plate.
   1.4. Cover the microassay plate and incubate at room temperature (20-25°C) for 60 ± 2 minutes with uniform shaking.
   1.5. Remove cover and wash each strip 4 times with Wash Buffer (250μL-350μL/well).

2. **CONJUGATE INCUBATION**
   2.1 Add 100μL Conjugate/well.
   2.2 Again cover the microassay plate and incubate at room temperature (20-25°C) for 30 ± 2 minutes with uniform shaking.
   2.3 Wash each strip as in Step 1.5

3. **COLOR DEVELOPMENT**
   3.1. Add 100μL Substrate/well and incubate at room temperature for 15 minutes exactly.

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

5. **CALCULATE RESULTS**

REFERENCES


11. Kievit, J.K. et. al. (1997). Release of alpha-glutathione S-transferase (αGST) and pi-glutathione S-transferase (πGST) from ischemic damaged kidneys into the machine perfusate - relevance to viability assessment Transplantation Proceedings 29(8), 3591-3593.


