

**REF** **BIO60HEPAS**  
**96 well plate**



**ARGUTUS** **MEDICAL**

**High Sensitivity**  
**Alpha GST EIA**

**Enzyme Immunoassay**

**Instructions for Use**

**FOR RESEARCH USE ONLY**  
**Not for use in Diagnostic Procedures**

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## **INTENDED USE**

The Argutus Medical High Sensitivity Alpha GST EIA is a method for the quantitative determination of alpha glutathione S-transferase ( $\alpha$ GST) in biological fluids such as serum, plasma, bile, tissue homogenates and tissue culture supernatants.  $\alpha$ GST can be assayed in samples from humans<sup>1,2</sup> and non-human primates<sup>3,4</sup>.

For the assay of  $\alpha$ GST in other biological fluids, from other species or other GST subclasses, contact Argutus Medical for advice.

The Argutus Medical High Sensitivity Alpha GST EIA is for research use only.

## **BACKGROUND**

### **HEPATIC STUDIES**

In liver, alpha glutathione S-transferase is located in the hepatocytes whereas PiGST ( $\pi$ GST) is confined to the bile duct cells<sup>5-8</sup>. 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, hepatocyte injury is monitored by the measurement of 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<sup>5</sup>. In contrast,  $\alpha$ GST is present in high concentrations in both the centrilobular and periportal regions. Since the centrilobular hepatocytes are very susceptible to damage in a variety of conditions, including allograft rejection<sup>9</sup>, viral hepatitis<sup>10</sup>, chronic active hepatitis<sup>11</sup>, and hepatotoxicity<sup>12</sup>,  $\alpha$ GST is a more sensitive indicator of acute hepatocyte injury in these and other conditions.

The Argutus Medical High Sensitivity Alpha GST EIA is a sensitive, specific, precise immunoassay for  $\alpha$ GST and, being an EIA, is unaffected by inhibitors of enzyme activity (e.g. bilirubin<sup>1</sup>).

### **CELL CULTURE**

$\alpha$ GST release is a sensitive *in-vitro* indicator of injury to hepatocytes and renal proximal tubular cells<sup>13</sup>.

## **ASSAY PRINCIPLE**

Argutus Medical High Sensitivity Alpha GST is a quantitative enzyme immunoassay. The test procedure is based on the sequential addition of sample, enzyme-conjugate and substrate to microassay wells coated with anti- $\alpha$ GST. The resultant colour intensity is proportional to the amount of  $\alpha$ GST present in the sample. The assay range is 62.5-2000ng/L.

## **COMPONENTS**

Each Argutus Medical High Sensitivity Alpha GST EIA kit contains reagents sufficient for 96 assay wells, equivalent to one calibration curve and 41 samples in duplicate

1. Antibody coated Microassay plate: 

|     |
|-----|
| PLA |
|-----|

  
96 wells (12x8 break well strips) coated with IgG directed against  $\alpha$ GST  
READY TO USE
  
2.  $\alpha$ GST Calibrator 4mg/L: 

|     |
|-----|
| CAL |
|-----|

  
Purified  $\alpha$ GST in stabilising buffer (200 $\mu$ L)  
Contains Thiomersal and sodium azide  
STOCK SOLUTION
  
3. Positive Control: 

|         |   |     |
|---------|---|-----|
| CONTROL | + | 10X |
|---------|---|-----|

  
10X Protein containing solution with  
added stabilisers (4.5mL)  
Contains Thiomersal and sodium azide  
CONCENTRATE
  
4. Conjugate Concentrate: 

|      |     |
|------|-----|
| CONJ | 10X |
|------|-----|

  
10X Anti- $\alpha$ GST IgG conjugated to horseradish peroxidase (1.4mL)  
Contains Thiomersal  
CONCENTRATE
  
5. Wash Concentrate: 

|     |      |     |
|-----|------|-----|
| BUF | WASH | 20X |
|-----|------|-----|

  
20X Phosphate buffered saline /  
Tween-20 (PBST, 55mL)  
Contains Thiomersal  
CONCENTRATE
  
6. Substrate: 

|      |     |
|------|-----|
| SUBS | TMB |
|------|-----|

  
Stabilised TMB solution (11mL)  
READY TO USE
  
7. Stop Solution: 

|      |     |
|------|-----|
| SOLN | STP |
|------|-----|

  
1N Sulphuric Acid (11mL)  
READY TO USE
  
8. Instructions for use 

|     |
|-----|
| INS |
|-----|

## **PRECAUTIONS**

### **SAFETY**

- The Argutus Medical High Sensitivity Alpha GST EIA kit is for research use only and is not for use in diagnostic procedures.
- The Argutus Medical High Sensitivity Alpha GST EIA kit is intended for use by qualified laboratory staff only.
- The kit contains material of human origin, which has been tested and found to be negative for Hepatitis B DNA, HCV RNA and HIV RNA. However, since no test can provide complete assurance, treat all materials as potentially infectious.
- Some reagents contain Thiomersal which may be toxic if ingested.
- The Stop Solution contains sulphuric acid, which is corrosive and causes burns. 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.
- Some reagents contain sodium azide which may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagent should be flushed with large volumes of water to prevent azide build up.
- Dispose of all infected or potentially infected material in accordance with good laboratory practice. All such materials should be treated as potentially infectious.
- Residues of chemicals 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.

**WARNING:** This product contains a chemical known to the State of California to cause birth defects or other reproductive harm (California Prop 65: Thiomersal).

### **PROCEDURAL**

- Do not use kit, or individual reagents, which are past their expiry date.
- Do not mix or substitute reagents from kits with different lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges specified may produce invalid results. Assays that do not fall 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 have precipitated out of solution.
- Ensure Wash Concentrate is mixed thoroughly and no crystals remain before reconstitution
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour 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.
- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural 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 expiry date shown.
2. αGST Calibrators must be used within 30 minutes of preparation.
3. Diluted positive control must be used within 30 minutes of preparation.
4. Prepared wash solution (PBST) is stable at room temperature for up to two weeks. Store at 2-8°C if extended storage is required.
5. Diluted conjugate must be used within 1 hour of preparation.
6. Plate assay wells should be stored in sealed bags with desiccants at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.

## **ADDITIONAL MATERIALS REQUIRED**

1. Micropipettes (5µL to 50µL, 50µL to 200µL, 200µL to 1000µL) and a multichannel pipette (50µL to 200µL)
2. Microassay strip washing system
3. ELISA plate reader capable of reading at 450nm (using 630nm as reference if possible)
4. 1L beaker
5. Timer
6. Liquid trough
7. Deionised/distilled water
8. Plate shaker
9. Graduated cylinder
10. Test tubes

## **PREPARATION OF REAGENTS**

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

### **WASH SOLUTION (PBST)**

Prepare a 1/20 dilution of wash concentrate by adding, for example, 10mL wash concentrate to 190mL deionised water as required. 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.

### **CALIBRATORS**

Prepare the 40µg/L working stock solution (A) from the αGST stock solution as follows:

|                 |                     |
|-----------------|---------------------|
| Stock Solution: | 25µL                |
| Wash Solution:  | <u>2500µL</u>       |
| Total:          | 2525µL @ 40µg/L (A) |

Using labelled test tubes, prepare calibrators as follows:

| <b>To Prepare Calibrator (ng/L)</b> | <b>Volumes of Solutions Required</b>  |
|-------------------------------------|---------------------------------------|
| 2000 (B)                            | Add 50µL of A to 950µL Wash solution  |
| 1000 (C)                            | Add 500µL of B to 500µL Wash solution |
| 500 (D)                             | Add 500µL of C to 500µL Wash solution |
| 250 (E)                             | Add 500µL of D to 500µL Wash solution |
| 125 (F)                             | Add 500µL of E to 500µL Wash solution |
| 62.5 (G)                            | Add 500µL of F to 500µL Wash solution |
| 0 (H)                               | 500µL Wash solution                   |

### **CONJUGATE**

Prepare a 1/10 dilution of Conjugate Concentrate by adding, for example, 100µL Conjugate Concentrate to 900µL Wash Solution. Each strip of 8 microassay wells requires a minimum of 1000µL of diluted Conjugate.

### **POSITIVE CONTROL**

Prepare a 1/10 dilution of Positive Control by adding 50µL Positive Control to 450µL Wash Solution.

## **SPECIMEN HANDLING AND STORAGE**

Serum samples are stable at 2-8°C for 3 days but 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 9 months. Repeated freeze-thawing of samples should be avoided in order to prevent loss of αGST. No significant differences have been observed between αGST levels in matched serum and plasma samples<sup>10</sup>.

## **SAMPLE PREPARATION**

Immediately prior to the assay, dilute samples and the Positive Control 1/10 by adding 50µL sample to 450µL wash solution

## **ASSAY PROCEDURE**

**Note:** To obtain precise reproducible results, it is essential that care be taken with the washing steps. The following points should be noted:

- Fill wells evenly and aspirate completely.
- At the end of the last wash step, remove any remaining drops by tapping the microassay plate hard against paper towels until no more drops are remaining. Do not dry the inside of the wells.
- Add next reagent promptly.

### **1. SAMPLE/CALIBRATOR INCUBATION**

- 1.1 Prepare Wash Solution, Calibrators, Positive Control and Conjugate 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, 2 for each of the Controls and samples).  
Add Calibrators (**H-B; 0-2000µg/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.

**Note:** A lab-line instruments titer plate shaker was used – speed 2-3.

- 1.5 Remove cover and wash each strip 6 times with Wash Solution (**250-350µL/well**).  
When complete, firmly tap the plate against a paper towel to ensure complete removal of wash solution from wells.

**Note:** Either automated or manual washing is acceptable.

### **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 **60 ± 2 minutes** with uniform shaking.

**Note:** A lab-line instruments titer plate shaker was used – speed 2-3.

- 2.3 Wash each strip as in 1.5 above.

### 3. COLOUR DEVELOPMENT

3.1 Add **100µL** Substrate/well using a multichannel pipette and incubate at room temperature for 15 minutes exactly.

### 4. STOP

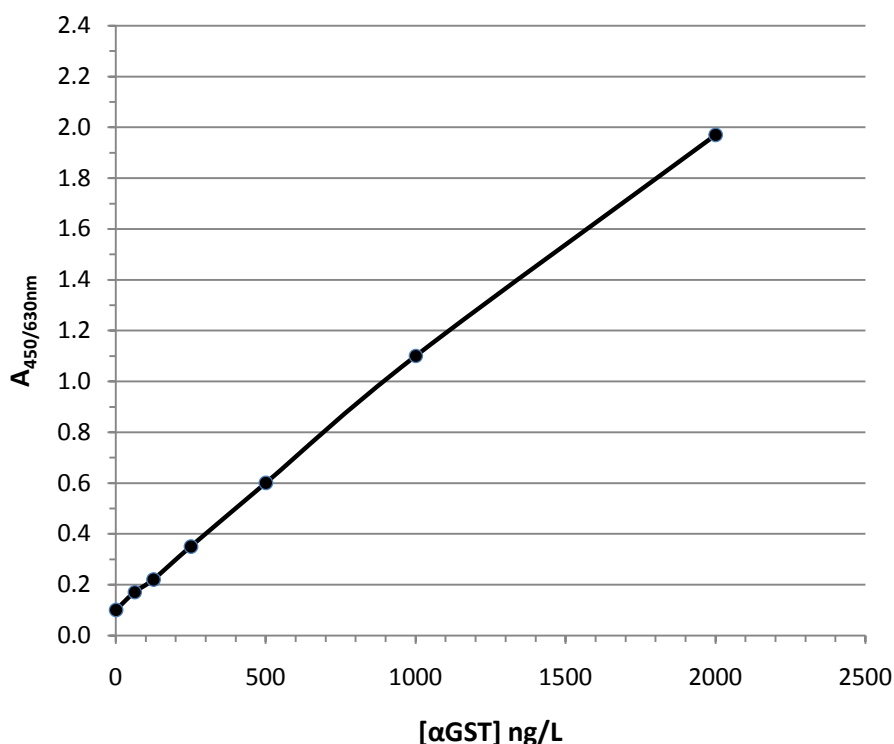
4.1 Stop the reaction by addition of **100µL** Stop solution/well.  
Ensure complete mixing of Substrate and Stop Solution

4.2 Read **immediately** 450nm with 630nm as reference (if available).

## CALCULATION OF RESULTS

1. Calculate the mean absorbance for each Calibrator, Positive Control and sample.
2. Plot a calibration curve of  $A_{450/630nm}$  versus  $[\alpha\text{GST}]\text{-ng/L}$  on a linear-linear scale (See Figure 1.)
3. Read the  $[\alpha\text{GST}]\text{ng/L}$  indicated by the mean absorbance of the samples and Positive Control from the calibration curve.
4. Multiply the calculated  $[\alpha\text{GST}]$  for samples by the appropriate dilution factor in order to obtain the actual  $[\alpha\text{GST}]$ .
5. Multiply the calculated  $[\alpha\text{GST}]$  for the control by 10 to obtain actual  $[\alpha\text{GST}]$  for the control. The concentration should be within the range given on the inside of the box lid.
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.

## **EXAMPLE OF A CALIBRATION CURVE**



**Figure 1:** Typical calibration curve obtained using Argutus Medical High Sensitivity Alpha GST EIA. Plot of A<sub>450/630nm</sub> versus [αGST] ng/L.

## **PERFORMANCE CHARACTERISTICS**

### **MEASURING RANGE**

The calibration curve covers the range 62.5-2000ng/L equivalent to up to 20 000ng/L in samples diluted 1/10. This range may be extended by increasing sample dilution.

### **LIMIT OF DETECTION**

The detection limit of Argutus Medical High Sensitivity Alpha GST EIA is 9.1ng/L, equivalent to 91ng/L in a sample diluted 1/10.

### **SPECIFICITY**

Argutus Medical High Sensitivity Alpha GST EIA is highly specific for human αGST. No measurable cross reactivity is observed with human piGST or muGST, nor with porcine, rat or canine αGST.

## REPRODUCIBILITY

**Intra-assay Variation:** Samples were assayed in replicates of twenty on three occasions

| Sample     | [ $\alpha$ GST] ng/L | CV%   | n  |
|------------|----------------------|-------|----|
| High       | 11 600               | 11.06 | 60 |
| Medium     | 5 900                | 6.96  | 60 |
| Low-Medium | 3 620                | 9.66  | 60 |
| Low        | 2 210                | 10.76 | 60 |

**Inter-assay Variation:** Samples were assayed in duplicates on 36 occasions.

| Sample     | [ $\alpha$ GST] ng/L | CV%   | n  |
|------------|----------------------|-------|----|
| High       | 12 400               | 7.43  | 36 |
| Medium     | 5 360                | 14.23 | 36 |
| Low-Medium | 3 250                | 17.78 | 36 |
| Low        | 2 240                | 20.28 | 36 |

## RECOVERY

Mean recovery when  $\alpha$ GST was added over the range of 200 to 1600ng/L was 116.8%.

## WARRANTY

The performance data presented here was obtained using the procedure described. Any change or modification of the procedure, not recommended by Argutus Medical, may affect the results, in which case Argutus Medical disclaims all warranties, expressed, implied or statutory, including implied merchantability and fitness for use. In the case of such an event, Argutus Medical shall not be liable for damages, direct or consequential.

## **SUMMARY OF ASSAY PROCEDURE**

**Note: All incubations are performed at room temperature**

1. Pipette: **100 $\mu$ L standards/positive control/sample**



Incubate: **60 min**



Wash: **6 X 300 $\mu$ L**

2. Pipette: **100 $\mu$ L enzyme conjugate**



Incubate: **60 min**



Wash: **6 X 300 $\mu$ L**

3. Pipette: **100 $\mu$ L substrate**



Incubate: **15 min**

4. Pipette: **100 $\mu$ L stop solution**



Read immediately: **450nm/630nm**

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## **OTHER ARGUTUS MEDICAL ASSAYS**

### **Pancreatic Injury Testing Service**

| <b>Catalogue No</b> | <b>Product Name</b>                      | <b>Description</b>                          |
|---------------------|--|---|
| <b>TEST BBU</b>     | Trypsinogen Activation Peptide (TAP) EIA | TAP in human and mammalian urine and tissue |

### **Animal Organ Damage Biomarkers**

| <b>Catalogue No</b> | <b>Product Name</b> | <b>Description</b>                                  |
|---------------------|---------------------|---|
| <b>BIO64RT</b>      | Rat Alpha GST EIA   | $\alpha$ GST in rat serum, urine and tissue culture |
| <b>BIO76YB1</b>     | Rat Yb1 GST EIA     | GSTYb1 ( $\mu$ GST) in rat urine                    |
| <b>BIO87CD</b>      | RPA-1 Antibody      | Antibody to rat collecting duct                     |
| <b>BIO88LH</b>      | RPA-2 Antibody      | Antibody to rat loop of henle                       |

### **Human Organ Damage Biomarkers**

| <b>Catalogue No</b> | <b>Product Name</b>            | <b>Description</b>  |
|---------------------|--------------------------------|---|
| <b>BIO66NEPHA</b>   | NEPHKIT® Alpha GST EIA         | $\alpha$ GST in human urine   |
| <b>BIO60HEPA</b>    | HEPKIT® Alpha GST EIA          | $\alpha$ GST in human serum and plasma  |
| <b>BIO60HEPAS</b>   | High Sensitivity Alpha GST EIA | $\alpha$ GST in human serum and plasma  |
| <b>BIO85</b>        | Pi GST EIA                     | $\pi$ GST in human urine and plasma   |
| <b>BIO83</b>        | Urinary Collagen IV EIA        | Collagen IV in human urine  |
| <b>BIO82</b>        | Serum Collagen IV EIA          | Collagen IV in human serum  |
| <b>BIO81DNA</b>     | OxyDNA Test                    | Fluorescence method for the detection of oxidative DNA damage in cell suspensions |



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