

**REF** BIO60HEPA  
**96 Well Plate**



**ARGUTUS MEDICAL**

**HEPKIT®-Alpha  
Human Alpha GST**

**Enzyme Immunoassay**

**US ENGLISH**

**Instructions for Use**

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

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## **INTENDED USE**

The Argutus Medical HEPKIT®-Alpha kit provides a method for the quantitative determination of alpha glutathione S-transferase ( $\alpha$ GST) in serum and sodium-heparinised plasma. For the assay of other GST classes, please contact Argutus Medical for further information. The Argutus Medical HEPKIT® - Alpha Kit is for research use only in the USA.

## **BACKGROUND**

In liver, alpha glutathione S-transferase is located in the hepatocytes whereas pi GST ( $\pi$ GST) is confined to the intrahepatic bile duct cells<sup>1,2,3</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, 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<sup>4</sup>. In contrast,  $\alpha$ GST has been found to be equally distributed in both the centrilobular and periportal regions<sup>2,3</sup>. Since the centrilobular hepatocytes are very susceptible to damage in a variety of clinical conditions including Allograft Rejection<sup>5,6,7</sup>, Viral Hepatitis<sup>8</sup>, Chronic Active Hepatitis<sup>9</sup>, and Hepatotoxicity<sup>10</sup>,  $\alpha$ GST would be a more sensitive indicator of hepatic status in these, and other, clinical situations.

HEPKIT®-Alpha is a specific, precise immunoassay for  $\alpha$ GST<sup>11,12</sup> and, being an EIA, is unaffected by modulators of enzyme activity (e.g. bile salts and bilirubin)<sup>11</sup>. Thus, it is now possible to use  $\alpha$ GST quantitation to study the hepatocellular status of individuals at risk of hepatic damage.

## **ASSAY PRINCIPLE**

Argutus Medical HEPKIT®-Alpha 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 IgG. The resultant colour intensity is proportional to the amount of  $\alpha$ GST present in the sample. The assay range is 1.25-40 $\mu$ g/L.

## **COMPONENTS**

1. Antibody Coated Microassay Plate  
12x8 well strips coated with IgG directed against  $\alpha$ GST.  
Breakapart wells.  
READY TO USE

PLA
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2. GST Calibrator  
Purified  $\alpha$ GST in stabilising diluent (200 $\mu$ L).  
Contains Thiomersal and sodium azide.  
STOCK SOLUTION

CAL
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3. Positive Control  
 $\alpha$ GST in protein containing  
solution with added stabilisers (4.5mL).  
Contains Thiomersal and sodium azide.  
READY TO USE

CONTROL	+
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4. Conjugate Concentrate  
51x Anti- $\alpha$ GST IgG conjugated to  
horseradish peroxidase (300 $\mu$ L).  
Contains Thiomersal.  
CONCENTRATE

CONJ	51X
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5. Wash Concentrate  
20x Phosphate buffered  
saline/Tween-20 (PBST 55mL).  
Contains Thiomersal.  
CONCENTRATE

BUF	WASH	20X
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6. TMB substrate  
Stabilised liquid TMB  
solution (11mL).  
READY TO USE

SUBS	TMB
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7. Stop Solution  
0.5 mol/L Sulphuric acid (11mL).  
READY TO USE

SOLN	STP
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8. Instructions for use

INS
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## **PRECAUTIONS**

### **SAFETY**

- The Argutus Medical HEPKIT® Alpha Kit is for research use only ; not for use in diagnostic procedures.
- The Argutus Medical HEPKIT® Alpha 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 also 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.
- 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 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 product contains a chemical known to the State of California to cause birth defects or other reproductive harm (California Prop 65: Thiomersal).

### **PROCEDURAL**

- Argutus Medical recommend that for clinical trials projects, users assay all samples using the same kit lot number for optimal study consistency.
- Do not use kit or individual reagents past their expiry 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.

- 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 the expiry date shown.
2. αGST Calibrators must be used within 30 minutes of preparation.
3. Prepared Wash Solution (PBST) is stable at room temperature for up to two weeks and up to one month at 2-8°C.
4. Prolonged storage of diluted Conjugate at room temperature should be avoided. Use within 15 minutes of preparation.
5. Plate assay wells should be stored in sealed foil pouch 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 and 200µL to 1000µL) and a multichannel pipette (50µL to 200µL).
2. Microassay strip washing system.
3. ELISA plate reader capable of measuring at 450nm with reference at 630nm if available.
4. 1L beaker.
5. Timer.
6. Liquid trough.
7. Deionised/Distilled water.
8. Plate shaker.
9. Graduated cylinder.
10. Test tubes.

## **PREPARATION OF REAGENTS**

### **1. WASH SOLUTION (PBST)**

Perform a 1/20 dilution of Wash Concentrate 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.)

### **2. CALIBRATORS**

Prepare Calibrator (A) from the αGST stock solution as follows.

Stock:	25µL
Wash Solution:	<u>2500µL</u>
Total:	2525µL (A)

Using labelled tubes prepare further calibrators as follows:

<b>Equivalent Calibrator Concentration</b>	<b>Calibrator Volume (µL)</b>	<b>Wash Solution Volume (µL)</b>
40µg/L (A)	500 (A)	-
20µg/L (B)	500 (A)	500
10µg/L (C)	500 (B)	500
5µg/L (D)	500 (C)	500
2.5µg/L (E)	500 (D)	500
1.25µg/L (F)	500 (E)	500
0µg/L (G)	-	500

### **3. CONJUGATE**

Immediately prior to use, dilute the Conjugate Concentrate 1/51 by adding 20µL Conjugate to 1mL Wash Solution per Microassay. Each strip requires 1020µL of prepared conjugate.

## **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 to prevent loss of αGST. No significant differences have been observed between the recovery of αGST in sodium-heparinised plasma and serum.

## **SAMPLE PREPARATION**

Immediately prior to the assay, dilute samples 1/5 by adding 50µL sample to 200µL Wash Solution. 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. The Positive Control does not require dilution.

## **FULL ASSAY PROCEDURE (1)**

NOTE: All reagents should be allowed to reach room temperature 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 Microassay wells in the assay plate (14 for the Calibrators plus two each for the Controls and samples). Arrange in columns of 8 and fill up spaces in the columns with blank Microassay wells. Add Calibrators (G-A ; equivalent concentration 0-40µg/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.

### **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 (**250µL - 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.
- 2.3 Add **100µL** Conjugate/well.
- 2.4 Again cover the Microassay plate and incubate at room temperature (20-25°C) for **30 ± 2 minutes** with uniform shaking.  
Note: A Lab-line Instruments Titer Plate Shaker was used-Speed 2-3.
- 2.5 Wash each strip as in Step 2.2.

### **3. COLOUR DEVELOPMENT**

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

### **4. STOP**

- 4.1 Add **100µL** Stop Solution/well using a multi channel 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)**

1. Calculate the mean absorbance for each Calibrator, Control and Sample.
2. Plot a Calibration curve of  $A_{450/630nm}$  versus  $[\alpha\text{GST}]$  ( $\mu\text{g/L}$ ). The curve should have a similar shape to Figure 1.
3. Read the  $[\alpha\text{GST}]$  ( $\mu\text{g/L}$ ) indicated by the mean absorbances of the samples from the calibration curve.
4. Multiply the calculated  $[\alpha\text{GST}]$  by the appropriate dilution factor in order to obtain the actual  $[\alpha\text{GST}]$ .
5. The concentration of 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.

## **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 assay procedure and subsequent assays is recommended.
4. The HEPKIT®-Alpha Positive Control should be included in all assays to allow the operator to monitor interassay reproducibility.
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  $[\alpha\text{GST}]$  should be computed as described in 'Calculation of Results (2)' below.

## **ABBREVIATED ASSAY PROCEDURE (2)**

Note: (1) All reagents should be allowed to reach room temperature prior to commencement of assay (2) Both **TEMPERATURE** and **INCUBATION TIMES** for sample analysis must be identical with those used for the preparation of the calibration curve. (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 Microassay 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 Microassay wells. Add 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 instrument Titer plate Shaker was used-Speed 2-3.

### **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 (**250µL - 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.
- 2.3. Add **100µL** Conjugate/well.
- 2.4. Again cover the Microassay plate and incubate at room temperature (20-25°C) for **30 ± 2** minutes with uniform shaking.  
Note: A Lab-line instrument Titer Plate Shaker was used-Speed 2-3.
- 2.5. Wash each strip as in Step 2.2.

### **3. COLOUR DEVELOPMENT**

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

### **4. STOP**

- 4.1. Add **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 (2)**

1. Calculate mean absorbance for each sample and Control.
2. Read the [ $\alpha$ GST] ( $\mu\text{g/L}$ ), 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 [ $\alpha$ GST] by the appropriate dilution factor in order to obtain the actual [ $\alpha$ GST].
4. The concentration of the Positive Control is read directly from the curve.
5. 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 given on the inside of the box lid. If this criterion is not met the assay is considered invalid and must be repeated.

## **PERFORMANCE CHARACTERISTICS**

### **REFERENCE RANGES**

Reference ranges for αGST in healthy individuals have been determined in many studies. Rees G. W *et al*<sup>1</sup> reported that the upper limit of the reference range (logarithmic mean + 2SD) was of 11.4µg/L, based on a population of 219 healthy blood donors. Argutus Medical recommends that all laboratories determine their own reference ranges.

### **LIMIT OF DETECTION**

The sample detection limit of Argutus Medical HEPKIT®-Alpha is 0.05µg/L in the Microassay well, 0.25µg/L in the sample.

### **MEASURING RANGE**

The calibration curve range covers 1.25-40µg/L, corresponding to 6.25-200µg/L in samples diluted 1/5 in Wash Solution. This range may be extended by increasing sample dilution.

### **SPECIFICITY**

HEPKIT®-Alpha 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 lipaemic, haemolytic or icteric samples. Lipaemic\*: Less than 10% interference up to 1000 IU in sample. Haemolytic: Less than 10% interference up to 1.17g/L haemoglobin in the sample. Icteric: Less than 11% interference up to 5mg/ml bilirubin in the sample.

\*Performed using intralipid 20% from Fresenius.

Some interference has been observed 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. Please contact Argutus Medical for further information.

### **REPRODUCIBILITY**

**Table 1:** Intra-assay variation of HEPKIT®-Alpha.

<b>Sample</b>	<b>[αGST] µg/L</b>	<b>SD</b>	<b>%CV</b>	<b>n</b>
Low	0.79	0.12	15.3	20
Medium	56.05	3.6	6.42	20
High	154.72	20.26	13.09	20

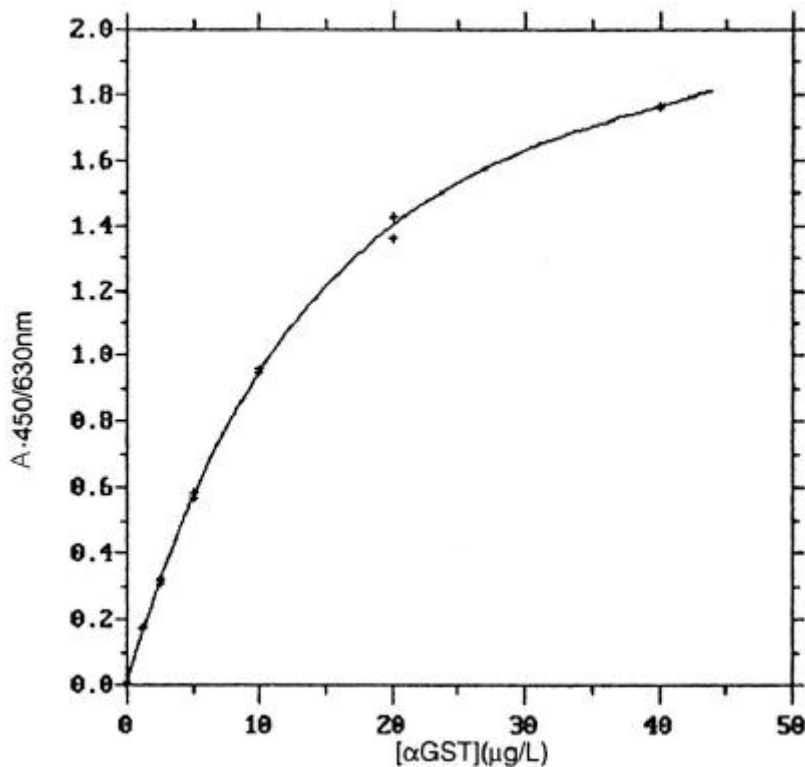
**Table 2:** Inter-assay variation of the HEPKIT®-Alpha using full assay procedure for each assay.

Sample	[αGST] µg/L	SD	%CV	n
Low	1.03	0.25	24.41	10
Medium	51.54	5.81	11.27	10
High	135.41	34.07	25.16	10
PC	12.84	1.12	8.68	10

**Table 3:** Inter-batch variation of the HEPKIT®-Alpha calculated across three batches of kits.

Sample	[αGST] µg/L	SD	%CV	n
Low	0.94	0.23	24.45	30
Medium	53.63	5.51	10.27	30
High	141.81	25.72	18.14	30

### EXAMPLE OF CALIBRATION CURVE



**Figure 1:** Typical Calibration curve obtained using Argutus Medical HEPKIT®-Alpha. Plot of  $A_{450/630nm}$  versus [αGST] µg/L. Assay range is 1.25-40µg/L αGST.

## **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**

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

- 1.1. Prepare Wash Solution and Calibrators.
- 1.2. Prepare Samples
- 1.3. Place Microassay wells in the assay plate. Add Calibrators, 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.

### **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 (**250µL - 350µL/well**).
- 2.3. Add **100µL** Conjugate/well.
- 2.4. Again cover the Microassay plate and incubate at room temperature (20-25°C) for **30 ± 2 minutes** with uniform shaking.
- 2.5. Wash each strip as in Step 2.2.

### **3. COLOUR DEVELOPMENT**

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

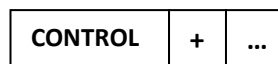
### **4. STOP**

- 4.1. Add **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**

## INTERPRETATION OF SYMBOLS

Positive Control Range



Batch code



Catalogue Number



Temperature limitation



Use by end of



Manufacturer



Biohazardous



## REFERENCES

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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	αGST in rat serum, urine and tissue culture
<b>BIO76YB1</b>	Rat Yb1 GST EIA	GSTYb1 (μGST) in rat urine
<b>BIO89RPA1</b>	RPA-1 EIA	Renal papillary antigen 1 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	αGST in human urine
<b>BIO85</b>	PI GST EIA	πGST in human urine and plasma
<b>BIO83</b>	Urinary Collagen IV EIA	Collagen IV in human urine
<b>BIO60HEPA</b>	HEPKIT® Alpha GST EIA	αGST in human serum and plasma
<b>BIO60HEPAS</b>	High Sensitivity Alpha GST EIA	αGST in human serum and plasma
<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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European Patent no. 640145  
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03/09