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ARGUTUS MEDICAL

Pi GST EIA

Enzyme Immunoassay

ENGLISH

Instructions for Use

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INTENDED USE

The Argutus Medical Pi GST EIA provides a method for the quantitative determination of Pi Glutathione S-transferase (π GST) in human plasma and urine. To assay π GST in other media and other GST subclasses, contact Argutus Medical for advice. The Argutus Medical Pi GST EIA is for research use only in the USA.

BACKGROUND

URINE STUDIES

π GST is located in the distal tubules of the human kidney whereas alpha GST (α GST) is confined mainly to the proximal tubules^{1, 2}. π GST is released into the urine of normal individuals as confirmed by enzyme immunoassay^{3, 4}. Any event which precipitates distal tubular damage may cause increased release of π GST into urine and elevations of urinary π GST levels have been shown to be indicative of distal tubule damage in renal transplant rejection^{5, 6} nephrotoxicity⁵⁻⁷, infection⁸, diabetes⁹ and chronic renal injury¹⁰. The release of α GST has been shown to be associated with proximal tubular damage, thus simultaneous measurement of α GST and π GST may allow discrimination between proximal and distal tubular damage⁵⁻⁷.

PLASMA STUDIES

Plasma levels of π GST have also been found to be elevated in chronic cholestatic diseases and cholangiocarcinoma¹¹. Elevated tissue and plasma π GST levels may be found in a range of malignancies¹².

ASSAY PRINCIPLE

Argutus Medical Pi GST 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- π GST IgG. The resultant colour intensity is proportional to the amount of π GST. The assay range is 1.25 - 40 μ g/L.

COMPONENTS

- | | | | | |
|--|--|---------|------|-----|
| 1. Antibody coated Microassay plate 96 wells (12x8 break well strips) coated with IgG directed against π GST. READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">PLA</td> </tr> </table> | PLA | | |
| PLA | | | | |
| 2. Calibrator Purified π GST in 50% (v/v) glycerol. (5mg/L, 100 μ L) Contains Thiomersal CONCENTRATE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">CAL</td> </tr> </table> | CAL | | |
| CAL | | | | |
| 3. Sample Diluent Protein containing solution with added stabilisers (50mL) Contains Sodium Azide READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">DIL</td> <td style="padding: 2px 10px;">SPE</td> <td style="padding: 2px 10px;">1X</td> </tr> </table> | DIL | SPE | 1X |
| DIL | SPE | 1X | | |
| 4. Wash Concentrate 20X Phosphate buffered saline / Tween-20 (PBST, 55mL) Contains Thiomersal CONCENTRATE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">BUF</td> <td style="padding: 2px 10px;">WASH</td> <td style="padding: 2px 10px;">20X</td> </tr> </table> | BUF | WASH | 20X |
| BUF | WASH | 20X | | |
| 5. Positive Control π GST (in protein containing solution with stabilisers (4.5mL)) Contains Thiomersal and sodium azide READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">CONTROL</td> <td style="padding: 2px 10px;">+</td> </tr> </table> | CONTROL | + | |
| CONTROL | + | | | |
| 6. Conjugate Anti- π GST IgG conjugated to horseradish peroxidase (11mL). Contains Thiomersal READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">CONJ</td> <td style="padding: 2px 10px;">ENZ</td> <td style="padding: 2px 10px;">1X</td> </tr> </table> | CONJ | ENZ | 1X |
| CONJ | ENZ | 1X | | |
| 7. Substrate Stabilised liquid TMB solution (11mL) READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">SUBS</td> <td style="padding: 2px 10px;">TMB</td> </tr> </table> | SUBS | TMB | |
| SUBS | TMB | | | |
| 8. Stop Solution 0.5M Sulphuric Acid (11mL). READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">SOLN</td> <td style="padding: 2px 10px;">STP</td> </tr> </table> | SOLN | STP | |
| SOLN | STP | | | |
| 9. NEPHKIT® Urine Stabilising Buffer (10mL) Contains Thiomersal and Sodium Azide READY TO USE | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">BUF</td> <td style="padding: 2px 10px;">NEPH</td> </tr> </table> | BUF | NEPH | |
| BUF | NEPH | | | |
| 10. Instructions for use | <table border="1" style="border-collapse: collapse;"> <tr> <td style="padding: 2px 10px;">INS</td> </tr> </table> | INS | | |
| INS | | | | |

PRECAUTIONS

SAFETY

- Argutus Medical Pi GST EIA is for *in-vitro* diagnostic use only.
- Argutus Medical Pi GST EIA 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. 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 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 mouth and never eat or drink at the laboratory workbench.

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.
- 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.

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. 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.
3. Pi GST Calibrators must be used within 30 minutes of preparation.
4. Prepared Wash Solution (PBST) is stable at room temperature for up to two weeks and for up to one month at 2-8°C.

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
11. Room temperature incubator

PREPARATION OF REAGENTS

1. WASH SOLUTION (PBST)

Perform 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 the Wash Concentrate at 37°C for 15-30 minutes will aid dissolution of salt crystals. Each strip of 8 wells requires 25mL Wash Solution.

2. CALIBRATORS

Prepare Calibrator (A) from the π GST stock solution as follows:

| | |
|-----------------|---------------------------------|
| Stock: | 20 μ L |
| Sample Diluent: | <u>2480μL</u> |
| Total: | 2500 μ L @ 40 μ g/L (A) |

Using labelled test tubes, prepare further calibrators as follows:

| Calibrator Concentration (μg/L) | Calibrator Volume (μL) | Volume Sample Diluent (μL) |
|---|--|--|
| 40 (A) | 500 (A) | 0 |
| 20 (B) | 500 (A) | 500 |
| 10 (C) | 500 (B) | 500 |
| 5 (D) | 500 (C) | 500 |
| 2.5 (E) | 500 (D) | 500 |
| 1.25 (F) | 500 (E) | 500 |
| 0 (G) | 0 | 500 |

SAMPLE COLLECTION

URINE

The Argutus Medical Pi GST EIA can be used to measure π GST in any urine sample but, due to the diurnal variation in proteinuria¹³, it is important for optimal results that timed, quantitative, urine samples are collected and the collection period and volume recorded. This will enable π GST excretion to be expressed as rate (ng/min). See Appendix 1. Overnight or 24 hour urine samples are recommended. For the use of other collection methods and periods, contact Argutus Medical for advice. As soon as possible after sample collection, add 200 μ L of NEPHKIT® Urine Stabilising Buffer to 800 μ L urine (4/5 dilution of sample), even if the samples are not to be stored. If, on visual inspection, the sample seems to contain blood, the sample must be immediately centrifuged at 10000g for 5 minutes. **After centrifugation, if the sample has a clear supernatant without signs of haemolysis, an aliquot can be collected and tested for π GST.** If, however, visual signs of blood are still present in the supernatant, the sample is unsuitable for π GST measurement. The presence of blood will not affect α GST measurements. The sample must be centrifuged, and the supernatant collected, prior to the addition of NEPHKIT® Urine Stabilising Buffer.

PLASMA

Maintain plasma at 2-8°C during all the following treatment. Collect samples in lithium heparin or EDTA tubes and centrifuge at 2500g for 10 minutes at 2-8°C within 6 hours of collection. Decant plasma supernatant carefully and recentrifuge this plasma at 6000g for 10 minutes at 2-8°C to ensure complete removal of platelets. Carefully collect plasma by aspiration taking care not to disturb any pellet. PiGST is present in platelets and their removal is important.

SAMPLE STORAGE

URINE

Do not store samples without the addition of NEPHKIT® Urine Stabilising Buffer. NEPHKIT® Urine Stabilising Buffer 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 NEPHKIT® Urine Stabilising Buffer, samples can be stored for one week at 2-8°C or at -20°C for at least 12 months. Repeated freeze thawing should be avoided. In the absence of NEPHKIT® Urine Stabilising Buffer, freezing can reduce GST levels in urine by up to 70% as measured by EIA. This decline in urinary GST is most likely due to denaturation during the freeze-thaw cycle.

PLASMA

Samples should be frozen at -20°C as soon as possible after collection. No change in π GST levels is observed in samples that have been stored at -20°C for up to 3 months. Repeated freeze-thawing of samples should be avoided.

SAMPLE PREPARATION

URINE

Immediately prior to the assay, dilute samples 1/2 by adding 200 μ L Sample to 200 μ L Sample Diluent.

PLASMA

Immediately prior to the assay, dilute samples 1/5 by adding 50 μ L Sample to 200 μ L Sample Diluent.

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 microassay plate with appropriate volume.

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 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 (available from Argutus Medical). 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.
- 1.5 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 the wells.
Note: Either automated or manual washing is acceptable.

2. CONJUGATE INCUBATION

- 2.1 Add **100µL** conjugate / well to the microassay plate.
- 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 Step 1.5.

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 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 $[\pi\text{GST}] \mu\text{g/L}$ (linear plot). (See Figure 1)
3. Read the $[\pi\text{GST}] (\mu\text{g/L})$ indicated by the mean absorbances of the Samples from the calibration curve.
4. Multiply the calculated $[\pi\text{GST}]$ by the appropriate dilution factor in order to obtain the actual $[\pi\text{GST}]$. Results for urine samples should be multiplied by an additional 1.25 to compensate for the dilution of sample by NEPHKIT® Urine Stabilizing Buffer.
5. The concentration for the Positive Control is read directly from the curve. Its value should be within the range given on the inside of the box lid.
6. For instructions on how to express urinary πGST release as rate (ng/min), see Appendix 1.
7. 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 should be considered invalid and must be repeated.

LIMITATIONS OF USE

Results must be correlated with the subject's clinical profile and other clinical laboratory results.

PERFORMANCE CHARACTERISTICS

REFERENCE RANGE

It is recommended that each laboratory develop a reference range relevant to its study group.

SPECIFICITY

The Argutus Medical Pi GST EIA is highly specific for π GST. No significant cross reactivity is observed with either mu or alpha isoforms of GST.

MEASURING RANGE

The calibration curve range covers 1.25-40 μ g/L, corresponding to 3.12-100 μ g/L in stabilized urine samples diluted 1/2 in Sample Diluent or 6.25-200 μ g/L in plasma samples diluted 1/5 in Sample Diluent. This range may be extended by increasing sample dilution.

LIMIT OF DETECTION

The detection limit of the Argutus Medical Pi GST EIA kit is 0.7 μ g/L. The limit of detection is 1.75 μ g/L in a stabilised urine sample diluted 1/2 or 3.5 μ g/L in a plasma sample diluted 1/5.

INTERFERENCE

No significant interference has been observed in this assay with lipaemic or icteric samples.

Lipaemia*: Less than 10% interference up to 1000IU in plasma sample.

Icteric: Less than 10% interference at 5mg/mL bilirubin in plasma samples and less than 10% at 5mg/mL bilirubin in urine samples.

Samples containing haemolysed blood are unsuitable for π GST measurements and must not be used.

*Performed using intralipid 20% from Fresenius.

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 the Argutus Medical Pi GST EIA.

| Sample | π GST (μ g/L) | SD | %CV | N |
|------------|------------------------|------|-----|----|
| Low plasma | 210.1 | 4.65 | 2 | 24 |
| Low urine | 3.8 | 0.09 | 2 | 24 |

Table 2: Inter assay variation of the Argutus Medical Pi GST EIA determined for 4 samples over 25 assays for one batch

| Sample | π GST(μ g/L) | SD | %CV | N |
|---------------|-----------------------|-------|-----|----|
| Low plasma | 218.2 | 14.72 | 7 | 25 |
| Medium plasma | 315.9 | 22.36 | 7 | 25 |
| Low urine | 5.4 | 0.52 | 9 | 25 |
| Low med urine | 61.5 | 3.56 | 6 | 25 |

Table 3: Inter batch variation of the Argutus Medical Pi GST EIA determined for 5 samples assayed 10 times with each of three batches.

| Sample | π GST(μ g/L) | SD | %CV | N |
|---------------|-----------------------|-------|-----|----|
| Low plasma | 222.4 | 13.35 | 6 | 30 |
| Medium plasma | 326.4 | 25.49 | 8 | 30 |
| Low urine | 6.1 | 1.00 | 16 | 30 |

EXAMPLE OF A CALIBRATION CURVE

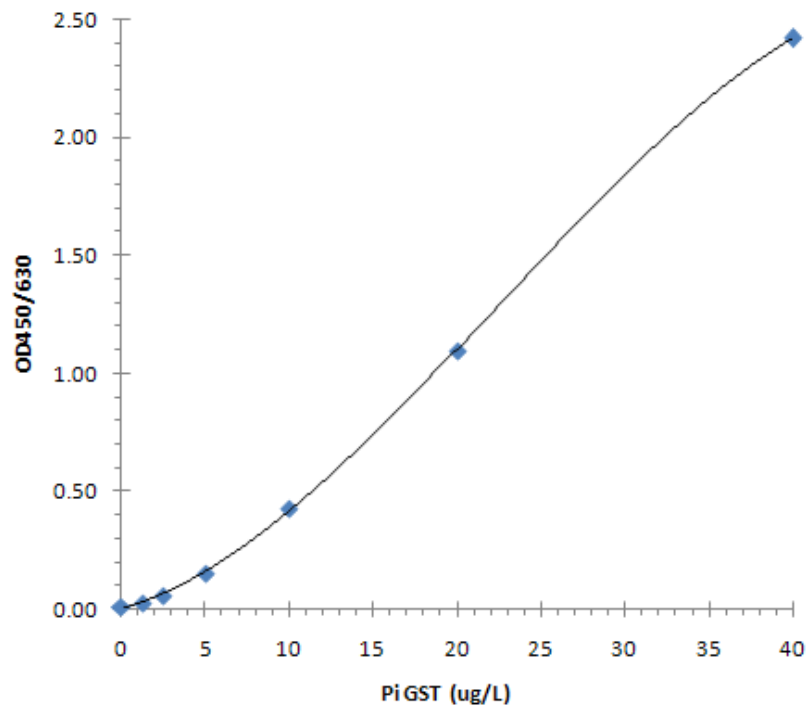


Figure 1: Typical Calibration Curve obtained using the Argutus Medical Pi GST EIA. Lin-lin plot of $A_{450/630\text{nm}}$ Versus $[\pi\text{GST}]\mu\text{g/L}$.

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.

APPENDIX 1

EXPRESSING π GST RELEASE AS RATE

Excretion of π GST is constant with time, not urine volume. This means that it may be more relevant to express π GST 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 π GST EXCRETION RATE

1. Determine urinary π GST levels using the Argutus Medical Pi GST EIA (μ g/L).
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:

$$\text{ng } \pi\text{GST/min} = \frac{\pi\text{GST}\mu\text{g/L} \times V}{T}$$

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 wells.
- 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 Solution. (**250-350µL / well**)

2. CONJUGATE INCUBATION

- 2.1. Add **100µL** Conjugate/well.
- 2.2. Cover the Microassay plate and incubate at room temperature (20-25°C) for **60 ± 2 minutes** with uniform shaking.
- 2.3. Wash each strip as in Step 1.5.

3. COLOUR DEVELOPMENT

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

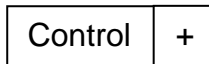
4. STOP

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

CALCULATE RESULTS

INTERPRETATION OF SYMBOLS

Positive control range



In vitro diagnostic medical device



Batch code



Catalogue Number



Temperature limitation



Use by end of



Manufacturer



Biohazardous



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OTHER ARGUTUS MEDICAL ASSAYS

Pancreatic Injury Testing Service

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

Animal Organ Damage Biomarkers

| Catalogue No | Product Name | Description |
|---------------------|---------------------|---|
| BIO64RT | Rat Alpha GST EIA | α GST in rat serum, urine and tissue culture |
| BIO76YB1 | Rat Yb1 GST EIA | GSTYb1 (μ GST) in rat urine |
| BIO89RPA1 | RPA-1 EIA | Renal papillary antigen 1 in rat urine |
| BIO87CD | RPA-1 Antibody | Antibody to rat collecting duct |
| BIO88LH | RPA-2 Antibody | Antibody to rat loop of henle |

Human Organ Damage Biomarkers

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



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Document Code: PI-284-ROW-09
05/09