REF BIO85
96 Well Plate

ARGUTUS MEDICAL

Pi 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 Pi GST EIA provides a method for the quantitative determination of Pi Glutathione S-transferase (πGST) in human urine. To assay πGST in other media and other GST subclasses, contact Argutus Medical for advice.

BACKGROUND

URINE STUDIES

πGST is located in the distal tubules of the human kidney whereas alpha GST (αGST) is confined mainly to the proximal tubules[1, 2]. πGST 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 πGST into urine and elevations of urinary πGST levels have been shown to be indicative of distal tubule damage in renal transplant rejection[5,6] nephrotoxicity[5-7], infection[8], diabetes[9] and chronic renal injury[10]. The release of αGST has been shown to be associated with proximal tubular damage, thus simultaneous measurement of αGST and πGST may allow discrimination between proximal and distal tubular damage[5-7].

ASSAY PRINCIPLE

Argutus Medical Pi GST EIA 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-πGST IgG. The resultant colour intensity is proportional to the amount of πGST. The assay range is 1.25 - 40µg/L.

COMPONENTS

1. Antibody coated Microassay plate
   96 wells (12x8 breakapart well strips coated with IgG directed against πGST).
   READY TO USE

   PLA

2. Calibrator, 0.1mL (5mg/L)
   Purified πGST in stabilising diluent containing ProClin 950 and Bronidox L as preservatives.
   125X CONCENTRATE

   CAL

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

   DIL | SPE | 1X

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

   BUF | WASH | 25X

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

   CONTROL | +

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

   CONJ | EN | 1X

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

   SUBS | TMB

8. Stop Solution
   0.5M Sulphuric Acid.
   READY TO USE

   SOLN | STOP

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

   BUF | USB

10. Instructions for use

    INS

PRECAUTIONS

SAFETY

- Argutus Medical Pi GST EIA is for research only use and not for use in diagnostic procedures.
- Argutus Medical Pi GST EIA 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 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.
- The components containing ProClin 950 are classified as per applicable European Community (EC) directives as: Irritant (Xi). The following are 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 past 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 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. Microassay wells should be stored in the sealed foil pouch with desiccants at 2-8ºC until required for use. Return unused wells to the storage pouch together with desiccants.
3. Pi GST Calibrators must be used within 30 minutes of preparation.
4. Prepared Wash Solution (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 ShakerRoom temperature incubator
10. Vortex

PREPARATION OF REAGENTS

1. WASH SOLUTION (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 Solution required for the assay. Each strip of 8 wells requires 25mL Wash Solution.

2. CALIBRATORS

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

Stock:	20μL
Sample Diluent:	2480μL
Total:	2500μL @ 40μg/L (A)

Mix Calibrator (A) by vortexing for 5 – 10 seconds. Using labelled test tubes, prepare further calibrators as follows:

πGST Calibrator Concentration (μg/L)	Calibrator Volume (μL)	Sample Diluent Volume (μL)
40 (A)	300 (A)	0
20 (B)	300 (A)	300
10 (C)	300 (B)	300
5 (D)	300 (C)	300
2.5 (E)	300 (D)	300
1.25 (F)	300 (E)	300
0 (G)	0	300

SAMPLE COLLECTION

Argutus Medical Pi GST EIA can be used to measure πGST in any urine sample but, due to the diurnal variation in proteinuria[11], it is important for optimal results that timed, quantitative, urine samples are collected and the collection period and volume recorded. This will enable πGST 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 Argutus Medical for advice.

As soon as possible after sample collection, add 100μL of Urinary Stabilising Buffer to 400μL 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 10000 x *g* for 5 minutes. **After centrifugation, if the sample has a clear supernatant without signs of haemolysis, an aliquot can be collected and tested for πGST**. If, however, visual signs of blood are still present in the supernatant, the sample is unsuitable for πGST measurement. The presence of blood will not affect αGST measurements. The sample must be centrifuged, and the supernatant collected, prior to the addition of Urinary Stabilising Buffer.

SAMPLE HANDLING AND STORAGE

Do not store samples without the addition of Urinary 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 should be avoided to prevent loss of πGST (up to 37% loss of πGST observed after 4 freeze-thaw cycles as measured by EIA).

SAMPLE PREPARATION

URINE

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

NOTE: If multiple sample additions (>10 duplicate samples) are to be undertaken then, to facilitate transfer to the assay plate, samples may 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 each for the positive controls and samples). 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 (350 ± 10rpm).

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 using a multichannel pipette.

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

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 in the dark for 15 minutes exactly with NO shaking.

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 [πGST] µg/L (4-parameter plot, refer to Figure 1).
3. Read the [πGST] (µg/L) indicated by the mean absorbances of the samples from the calibration curve.
4. Multiply the calculated [πGST] by the appropriate dilution factor in order to obtain the actual [πGST]. Results for stabilised urine samples should be multiplied by an additional 1.25 to compensate for the dilution of sample with Urinary 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 given 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.

LIMITATIONS OF USE

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

REFERENCE RANGE

Urine samples were obtained from apparently healthy donors without any clinical abnormal indications. πGST levels were determined using the Argutus Medical Pi GST EIA in order to establish the πGST concentration in the normal population.

The reference interval (5^{th} to 95^{th} percentiles) for Argutus Medical Pi GST EIA is 0 – 30.0μg/L in urine (n=132). This reference interval reflects the donor population of this study group. It is recommended that each laboratory determine their own reference range appropriate for their study group.

PERFORMANCE CHARACTERISTICS

MEASURING RANGE

The calibration curve range covers 1.25 - 40μg/L, corresponding to 3.13 - 100μg/L in stabilised urine samples diluted 1/2 in Sample Diluent. This range may be extended by increasing sample dilution.

PRECISION

A 20-day precision study was performed on the Argutus Medical Pi GST EIA based on guidance from the Clinical and Laboratory Standards Institute (CLSI) Document EP15-A2. Testing was performed on site using two lots of Argutus Medical Pi GST EIA and 4 different operators. Three urine pools containing endogenous πGST and three control samples spiked with πGST were assayed in duplicate at two separate times per day for 20 days. The data is summarized in the table below:

Sample	n	Mean [πGST] μg/L	Repeatability		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Urine Control	80	**10.2**	0.26	2.6	0.95	9.3	0.16	1.6	1.00	9.7
Medium Urine Control	80	**26.6**	0.78	2.9	1.71	6.4	0.88	3.3	2.08	7.8
High Urine Control	80	**49.0**	1.38	2.8	3.57	7.3	0.00	0.0	3.83	7.8
Low Urine Pool	80	**3.9**	0.22	5.6	0.41	10.4	0.15	3.9	0.49	12.4
Medium Urine Pool	80	**79.5**	2.23	2.8	6.83	8.6	0.00	0.0	7.18	9.0
High Urine Pool	80	**136.5**	2.92	2.1	12.63	9.3	3.48	2.6	13.42	9.8

SPECIFICITY

Argutus Medical Pi GST EIA is highly specific for πGST. No cross-reactivity was observed with μGST at 500μg/L or αGST at 500μg/L.

SENSITIVITY

The limit of detection (LoD) of Argutus Medical Pi GST EIA 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 1.3μg/L, which corresponds to a concentration of 3.25μg/L in a stabilised urine sample diluted 1/2.

LINEARITY UPON DILUTION

A urine sample pool containing a high level of endogenous πGST was diluted with Argutus Medical Pi GST EIA Sample Diluent to concentrations that spanned the range of the calibration curve and assayed on two lots of Argutus Medical Pi GST EIA. % Recovery was calculated as (Measured [πGST] μg/L / Expected [πGST] μg/L) x 100. The data obtained from testing on each kit lot is summarized in the table below:

Relative Dilution	Expected πGST (μg/L)	Measured πGST (μg/L)	% Recovery
100%	N/A	34.6	N/A
90%	31.1	30.9	99%
80%	27.7	27.2	98%
70%	24.2	24.3	100%
60%	20.8	20.4	98%
50%	17.3	17.6	102%
40%	13.8	13.7	99%
30%	10.4	10.1	98%
20%	6.9	6.9	99%
10%	3.5	3.3	96%
5%	1.7	1.6	95%
2.5%	0.9	1.0	113%

Relative Dilution	Expected πGST (μg/L)	Measured πGST (μg/L)	% Recovery
100%	N/A	34.6	N/A
90%	31.2	31.8	102%
80%	27.7	29.3	106%
70%	24.2	26.0	107%
60%	20.8	22.4	108%
50%	17.3	18.7	108%
40%	13.8	14.7	106%
30%	10.4	10.8	104%
20%	6.9	6.8	98%
10%	3.5	3.2	92%
5%	1.7	1.5	88%
2.5%	0.9	0.8	89%

SPIKE RECOVERY

Three urine pools containing endogenous πGST were each spiked with πGST analyte at three different concentrations and assayed using Argutus Medical Pi GST EIA. Recovery was calculated as (Measured [πGST] μg/L / Expected [πGST] μg/L) x 100. Data is presented in the following Table:

Urine	Spike Level	Measured πGST (μg/L) Final Conc.	Expected πGST (μg/L) Final Conc.	% Recovery
Low Urine Pool	Low	28.0	27.7	101%
	Medium	124.8	127.7	98%
	High	252.8	252.7	100%
Medium Urine Pool	Low	79.2	82.0	97%
	Medium	177.6	182.0	98%
	High	337.2	307.0	110%
High Urine Pool	Low	110.4	118.5	93%
	Medium	208.8	218.5	96%
	High	324.0	343.5	94%

INTERFERENCE

Potentially interfering endogenous substances were evaluated to determine their effect on πGST recovery using Argutus Medical Pi GST EIA. The potentially interfering substances listed below were spiked into a urine pool containing endogenous πGST at a concentration of ~65μg/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:

$$\% \text{ Bias: } \left[\frac{[\pi\text{GST}]\ \mu\text{g/L interferent-spiked urine}}{[\pi\text{GST}]\ \mu\text{g/L non-spiked urine}} \times 100 \right] - 100$$

Interfering Substance	Interferent Conc. (mg/dL)	Interference (% Bias)
Bilirubin (conjugated)	20	0%
Bilirubin (unconjugated)	20	-2%
Haemoglobin*	200	-6%
Albumin	6000	6%
Lipid**	1000	-2%
Human IgG	4	-1%
Tamm-Horsfall Protein	5	-7%

* No significant interference was observed with urine spiked with 200mg/dL haemoglobin. However, haemolysed urine samples are not suitable for πGST determination due to the potential release of πGST from platelets in the sample[12]. Therefore measurements performed on haemolysed urine samples may not truly reflect πGST of distal tubular origin.

** Performed with 20% Intralipid

EXAMPLE OF A CALIBRATION CURVE

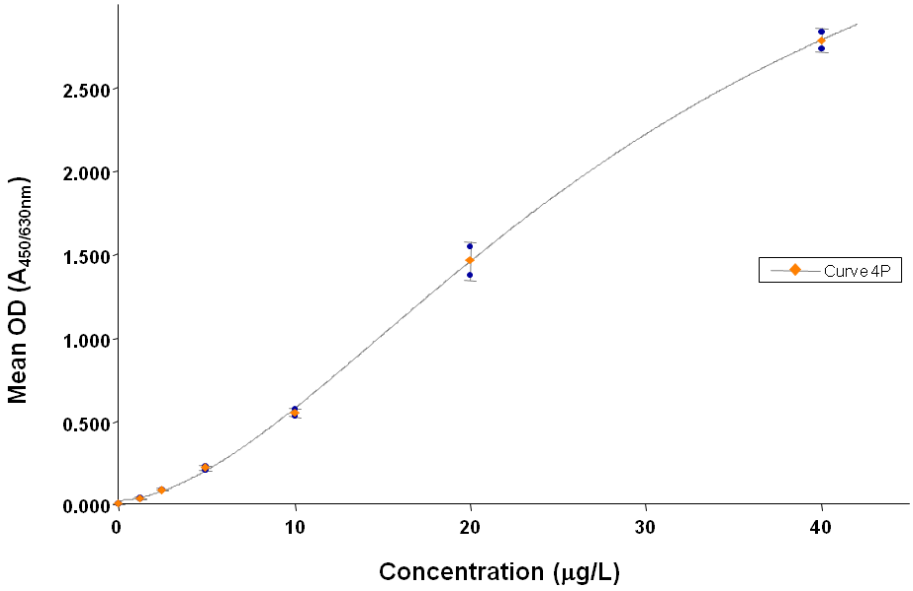

Figure 1: Typical calibration curve obtained using ARGUTUS MEDICAL Pi GST EIA. 4-parameter plot of $A_{450/630nm}$ Versus [πGST] μg/L. Assay range is 1.25 – 40 μg/L πGST.

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 πGST RELEASE AS RATE

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

1. Determine urinary πGST levels using the Argutus Medical Pi GST EIA (μg/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:

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

INTERPRETATION OF SYMBOLS

Positive control range	Control +
Batch code	LOT
Catalogue Number	REF
Temperature limitation	
Use by end of	
Manufacturer	
Biohazardous	

SUMMARY OF ASSAY PROCEDURE

1. SAMPLE / CALIBRATOR INCUBATION

1.1. Prepare Wash Buffer 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. Again, 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).

5. CALULATE RESULTS

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OTHER ARGUTUS MEDICAL ASSAYS

PRODUCTS FOR DETECTING HUMAN ORGAN DAMAGE

Catalogue No.	Product Description	Assay Format
H-RENA-E-001	Urinary KIM-1 EIA	96 Well EIA
H-RENA-E-005	Urinary KIM-1 EIA	5 x 96 Well EIA
H-RENA-25	Urinary KIM-1 Rapid Test	25 strips
H-RENA-50	Urinary KIM-1 Rapid Test	50 strips
Z-001	Urinary L-FABP EIA	96 Well EIA
BIO83	Urinary Collagen IV EIA	96 Well EIA
BIO85STB	Urine Stabilising Buffer	10 mL
BIO85STBC	Custom Filled Urine Stabilising Buffer Tubes	1 mL
BIO82	Serum Collagen IV EIA	96 Well EIA
BIO85	Human Pi GST EIA	96 Well EIA
BIO90NGAL	Human NGAL EIA	2 x 96 Well EIA
BIO81DNA	OxyDNA Test	50 Determinations
BIO84	Collagen IV Urine Collecting Tubes	

PRODUCTS FOR DETECTING RAT ORGAN DAMAGE

Catalogue No.	Product Description	Assay Format
R-RENA-E-001	Rat Urinary KIM-1 EIA	96 Well EIA
R-RENA-E-005	Rat Urinary KIM-1 EIA	480 Well EIA
R-RENA-25	Rat Urinary KIM-1 Rapid Test	25 strips
R-RENA-50	Rat Urinary KIM-1 Rapid Test	50 strips
BIO64RT	Rat Alpha GST EIA	96 Well EIA

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Document Code: PI-284-US-12
12/2011