

REF BIO66NEPHA
96 Well Plate



ARGUTUS MEDICAL

Nephkit[®] Alpha GST EIA

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 NEPHKIT[®] Alpha GST EIA provides a method for the quantitative determination of alpha glutathione S-transferase (α GST) in urine. For the assay of other GST classes, please contact Argutus Medical for further information. Argutus Medical NEPHKIT[®] Alpha GST EIA is for research use only in the USA.

BACKGROUND

Alpha Glutathione S-Transferase (α GST) is found in the proximal tubule region of the kidney whereas Pi Glutathione S-Transferase (π GST) is confined mainly to the distal tubules¹. α GST is released into the urine in normal individuals, as confirmed by immunoassay and western blot analysis². Any event which precipitates proximal tubule damage may cause increased release of α GST into urine and elevations of urinary α GST levels have been shown to be associated with proximal tubule damage. Urinary α GST has been found to be a valuable tool for studying proximal tubular injury in nephrotoxicity³⁻⁵, environmental toxicity⁶, surgery⁷, acute renal failure⁸ and transplantation⁹⁻¹².

The release of π GST has been shown to be associated with distal tubular damage⁶, thus simultaneous measurement of α and π GST may allow discrimination between proximal and distal tubular damage^{5,9-11} such as in nephrotoxicity⁵, acute renal failure⁸, transplant rejection⁹⁻¹⁰, ischaemia reperfusion injury⁹⁻¹¹ and diabetes¹³.

Urinary GSTs are sensitive indicators of current renal injury and may show renal effects while other biomarkers such as serum creatinine or blood urea nitrogen (BUN) are unchanged³⁻⁵.

ASSAY PRINCIPLE

Argutus Medical NEPHKIT[®] Alpha GST EIA 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- α GST IgG. The resultant color intensity is proportional to the amount of α GST present in the sample. The assay range is 1.25 - 40 μ g/L.

COMPONENTS

- | | | | | |
|---|--|---------|------|-----|
| <p>1. Antibody coated Microassay plate
12x8 well strips coated with IgG directed against αGST.
Breakapart wells.
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">PLA</td> </tr> </table> | PLA | | |
| PLA | | | | |
| <p>2. GST Calibrator
Purified αGST in stabilising diluent (200µL).
Contains Thiomersal and sodium azide.
STOCK SOLUTION</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">CAL</td> </tr> </table> | CAL | | |
| CAL | | | | |
| <p>3. Positive Control
αGST (in protein containing solution with
added stabilisers (4.5mL).)
Contains Thiomersal and sodium azide.
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">CONTROL</td> <td style="padding: 2px 10px;">+</td> </tr> </table> | CONTROL | + | |
| CONTROL | + | | | |
| <p>4. Conjugate Concentrate
51x Anti-αGST IgG conjugated to
horseradish peroxidase (300µL).
Contains Thiomersal.
CONCENTRATE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">CONJ</td> <td style="padding: 2px 10px;">51X</td> </tr> </table> | CONJ | 51X | |
| CONJ | 51X | | | |
| <p>5. Wash Concentrate
20x Phosphate Buffered/SalineTween-20
(PBST 55mL).
Contains Thiomersal.
CONCENTRATE</p> | <table border="1" style="margin-left: auto;"> <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 | | |
| <p>6. TMB substrate
Stabilized liquid TMB solution (11mL).
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">SUBS</td> <td style="padding: 2px 10px;">TMB</td> </tr> </table> | SUBS | TMB | |
| SUBS | TMB | | | |
| <p>7. Stop Solution
0.5 mol/L sulphuric acid (11mL).
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">SOLN</td> <td style="padding: 2px 10px;">STP</td> </tr> </table> | SOLN | STP | |
| SOLN | STP | | | |
| <p>8. Sample Diluent
Protein containing solution (50mL). Contains sodium azide.
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">DIL</td> <td style="padding: 2px 10px;">SPE</td> </tr> </table> | DIL | SPE | |
| DIL | SPE | | | |
| <p>9. NEPHKIT[®] Urine Stabilizing Buffer
Contains Thiomersal and sodium azide (10mL).
READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">BUF</td> <td style="padding: 2px 10px;">NEPH</td> </tr> </table> | BUF | NEPH | |
| BUF | NEPH | | | |
| <p>10. Instructions for use</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 2px 10px;">INS</td> </tr> </table> | INS | | |
| INS | | | | |

PRECAUTIONS

SAFETY

- The Argutus Medical NEPHKIT® Alpha GST EIA is for research use only; not for use in diagnostic procedures.
- The Argutus Medical NEPHKIT® 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. 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 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 recommends 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 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 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. α GST 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 for up to one month at 2-8°C.
4. Prolonged storage of diluted Conjugate at room temperature should be avoided. Use immediately upon preparation.
5. Plate assay wells should be stored in a sealed bag with desiccant at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.

ADDITIONAL MATERIALS REQUIRED

1. Micropipettes (5 μ L to 50 μ L, 50 μ L to 200 μ L, and 200 μ L to 1000 μ L) and a multichannel pipette (50 μ L to 200 μ L).
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 α GST stock solution.

Stock:	25 μ L
Sample Diluent:	<u>2500μL</u>
Total:	2525 μ L (A)

Using labeled test tubes prepare further calibrators as follows:

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

3. CONJUGATE

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

SAMPLE COLLECTION

Argutus Medical NEPHKIT[®] Alpha GST EIA can be used to measure α GST in any urine sample but, due to the diurnal variation in proteinuria¹⁴, it is important for optimal results that timed, quantitative, overnight urine samples are collected and the collection period and volume are recorded. This will enable urinary α GST release to be expressed as a rate (ng/min). See Appendix 1.

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 Stabilizing Buffer to 800 μ L urine (4/5 dilution of sample) even if the samples are not to be stored.

SAMPLE HANDLING AND STORAGE

Do not store samples without the addition of NEPHKIT[®] Urine Stabilizing Buffer. NEPHKIT[®] Urine Stabilizing 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 Stabilizing Buffer, samples can be stored 2-8°C for one week or at -20°C for up to 28 days.

Repeated freeze thawing should be avoided. In the absence of NEPHKIT[®] Urine Stabilizing 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.

SAMPLE PREPARATION

Immediately prior to the assay, dilute samples ½ by adding 200µL stabilized urine sample to 200µL Sample Diluent. 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 with appropriate volume adjustment. The Positive Control 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 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 concentrations 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 the wells.

Note: Either automated or manual washing 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 COLOR 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

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 [α GST] by the appropriate dilution factor in order to obtain the actual [α GST]. Results for samples should be multiplied by an additional 1.25 to compensate for dilution of sample NEPHKIT[®] Urine Stabilizing Buffer.
5. The concentration of the Positive Control is read directly from the curve.
6. For instructions on how to express α GST 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 must be considered invalid and must be repeated.

PERFORMANCE CHARACTERISTICS

REFERENCE RANGE

Timed quantitative overnight urine samples were collected from 38 healthy individual range from 18-46 years.

The observed urinary release of α GST was as follows:

As rate (ng/min)	
Mean	3.0 ng/min
Mean + 2SD	12.2 ng/min
As concentration (μ g/L)	
Mean	3.5 μ g/L
Mean + 2SD	11.1 μ g/L

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

LIMIT OF DETECTION

The sample detection limit of Argutus Medical NEPHKIT[®] Alpha GST EIA is 0.036 μ g/L in the Microassay well; equivalent to 0.09 μ g/L in the sample.

MEASURING RANGE

The standard curve covers 1.25-40µg/L, equivalent to 3.25-100µg/L in samples stabilized 4/5 in Urine Stabilizing Buffer and diluted 1/2 in Sample Diluent. This range may be extended by increasing sample dilution.

SPECIFICITY

Argutus Medical NEPHKIT[®] Alpha GST EIA is highly specific for the detection of αGST. No significant crossreactivity is observed with either mu or pi isoforms of GST.

INTERFERENCES

No significant interference has been observed in this assay with haemolytic and icteric samples. Haemolytic samples: Less than 14% interference with up to 1.17g/L haemoglobin in the sample. Icteric samples: Less than 11% interference with up to 5mg/mL bilirubin in the sample. In house studies have shown that urine samples with pH in the range 4-9 do not affect assay performance. Please contact Argutus Medical for further information.

REPRODUCIBILITY

Table 1. Intra-assay variation of Argutus Medical NEPHKIT[®] Alpha GST EIA.

Sample	[αGST] µg/L	SD	%CV	n
Low	9.03	0.65	7.18	20
Medium	33.4	2.48	7.43	20
High	59.1	5.47	9.24	20

Table 2. Inter-assay variation of the Argutus Medical NEPHKIT[®] Alpha GST EIA.

Sample	[αGST] µg/L	SD	%CV	n
Low	8.4	0.49	5.81	10
Medium	37.9	3.07	8.11	10
High	68.7	9.24	13.46	10
PC	10.8	1.35	12.53	10

Table 3. Inter-batch variation of the Argutus Medical NEPHKIT[®] Alpha GST EIA calculated across three batches of kits.

Sample	[αGST] µg/L	SD	%CV	n
Low	8.28	1.01	12.16	30
Medium	34.7	3.79	10.92	30
High	61.6	8.84	14.36	30

EXAMPLE OF CALIBRATION CURVE

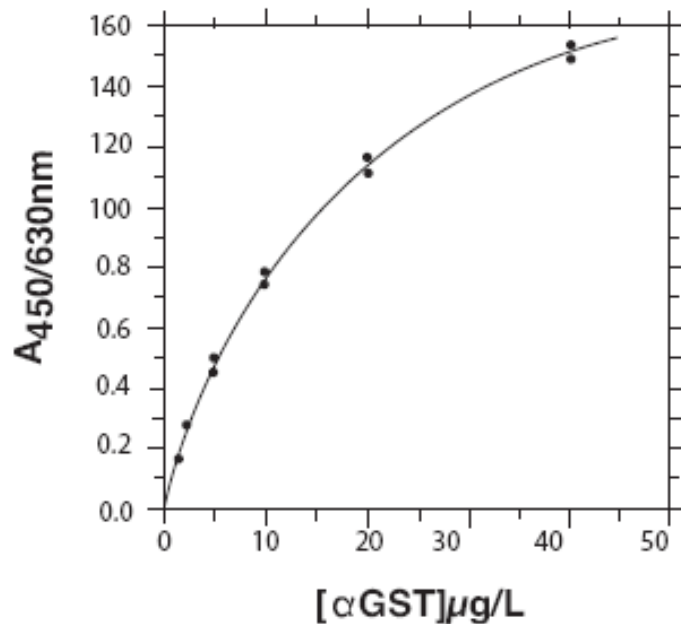


Figure 1: Typical Calibration curve obtained using Argutus Medical NEPHKIT[®] Alpha GST EIA. Plot of A_{450/630nm} versus [αGST] μg/L. Assay range is 1.25 - 40 μg/L αGST. Assay limit of detection is 0.036 μg/L in the Microassay well

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 Urinary αGST Release as Rate

The release 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 and Handling". Note the time of urination (T₂), time of the previous urination (T₁) and total urine volume (V).

CALCULATION OF RATE OF α GST RELEASE

1. Determine urinary α GST levels using Argutus Medical NEPHKIT[®] Alpha 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:

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

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 plates. Add Calibrators, Positive Control and diluted samples (100 μ L/well), in duplicate, to the Microassay plate.
- 1.4 Cover the Microassay plate and incubate at room temperature (20-25 $^{\circ}$ 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 (250 μ L-350 μ L/well).
- 2.3 Add 100 μ L Conjugate/well.
- 2.4 Again cover the Microassay plate and incubate at room temperature (20-25 $^{\circ}$ C) for 30 +/- 2 minutes with uniform shaking.
- 2.5 Wash each strip as in Step 2.2.

3. Color Development




- 3.1 Add 100 μ L Substrate/well and incubate at room temperature in the dark for 15 minutes exactly.

4. Stop

- 4.1 Add 100 μ L 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

INTERPRETATIONS OF SYMBOLS

Positive Control Range	<table border="1"><tr><td>CONTROL</td><td>+</td><td>...</td></tr></table>	CONTROL	+	...
CONTROL	+	...		
Batch code	<table border="1"><tr><td>LOT</td></tr></table>	LOT		
LOT				
Catalogue Number	<table border="1"><tr><td>REF</td></tr></table>	REF		
REF				
Temperature limitation				
Use by end of				
Manufacturer				

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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
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
BIO85	PI GST EIA	π GST in human urine 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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