REF BIO64RT
96 well plate

ARGUTUS MEDICAL
An EKF Diagnostics Company

Rat Alpha GST EIA

Enzyme Immunoassay

Instructions for Use

FOR RESEARCH USE ONLY
Not for use in Diagnostic Procedures

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INTENDED USE

Argutus Medical Rat Alpha GST EIA is an enzyme immunoassay for the quantitative estimation of rat alpha Glutathione S-Transferase (αGST) in biological fluids such as serum, urine and tissue culture supernatants.

BACKGROUND

HEPATIC STUDIES

αGST is a highly sensitive and specific biomarker of hepatocyte injury. The protein is found throughout the liver parenchyma in high concentrations[1, 2]. In the event of liver injury, αGST is released more rapidly than transaminases and has a shorter half-life in the circulation. Therefore, αGST levels more accurately indicate the onset and resolution of hepatocyte injury than transaminases[3,4]. αGST has been proven to be a superior indicator of hepatocyte injury in hepatotoxicity[3-5], transplantation[6,7] and ischemia-reperfusion injury[8]. αGST is a valuable marker for studying the use of extracorporeal support devices[9], where the use of the Argutus Medical rat αGST EIA kit enables the status of the host liver and experimental support device to be studied separately[9].

RENAL STUDIES

In the rat kidney, αGST is found in the proximal convoluted tubules[10]. It is readily and rapidly released into the urine when renal tubular injury occurs[11-13]. Urinary αGST levels correlate closely with the time course[11] and the severity of renal injury[12]. Urinary αGST levels are more sensitive indicators of renal tubular injury than serum creatinine[12].

ASSAY PRINCIPLE

The Argutus Medical Rat Alpha GST EIA is a quantitative solid phase enzyme immunoassay. The test procedure is based on the sequential addition of sample, antibody-enzyme conjugate and substrate to microassay wells coated with anti-rat αGST IgG. The resultant colour intensity is proportional to the amount of αGST in the sample. The assay range is 1.56-100µg/L.

COMPONENTS

Each Argutus Medical Rat Alpha GST EIA contains reagents for 96 assay wells, sufficient for 39 samples in duplicate.

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

   PLA

2. Enzyme Conjugate, 11mL
   Solution containing anti-rat αGST IgG conjugated to horseradish peroxidise with ProClin950 and Bronidox L as preservatives.
   READY TO USE

   CONJ

3. αGST Calibrator, 0.1mL (5.1mg/L)
   Purified rat αGST (YaYc isoform) in stabilising diluent containing ProClin950 and Bronidox L as preservatives
   Store at -20ºC until required
   51X STOCK SOLUTION

   CAL

4. Positive Control, 0.15mL
   Purified rat αGST in stabilising diluent containing ProClin950 and Bronidox L as preservatives
   Store at -20ºC until required
   50X STOCK SOLUTION

   CONTROL | +

5. Rat Urinary Stabilising Buffer, 10mL
   Contains ProClin950 and Bronidox L as preservatives
   READY TO USE

   BUF | NEPH

6. Sample Diluent, 55mL
   Protein containing solution with added stabilisers containing ProClin 950 and Bronidox L as preservatives
   READY TO USE

   DIL | SPE | 1X

7. Wash Buffer, 55mL
   Tris-buffered saline / Tween-20 (TBST) containing ProClin 950 as preservative.
   25X CONCENTRATE

   BUF | WASH | 25X

8. Substrate, 11mL
   Stabilised liquid TMB solution (11mL).
   READY TO USE

   SUBS | TMB

9. Stop Solution, 11mL
   0.5M Sulphuric Acid.
   READY TO USE

   SOLN | STP

10. Instructions for use

    INS

PRECAUTIONS

SAFETY

- The Argutus Medical Rat Alpha GST EIA kit is for research use only and is not for use in diagnostic procedures.
- The Argutus Medical Rat Alpha GST EIA kit is intended for use by qualified laboratory staff only.
- 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.
- 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.
- 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, 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 touch the sides of the wells 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 contain precipitates or that are cloudy in appearance.
- 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 bottom surface of the plate is clean and dry before reading.
- Before commencing the assay, an identification and distribution plan should be established.

- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural step if required.

STABILITY AND STORAGE

1. All kit reagents should be stored at 2-8°C **except for the αGST Calibrator and Positive Control stock solutions which should be stored at -20ºC on delivery.** All reagents are stable as supplied until the expiry date shown. The αGST Calibrator and Positive Control stock solutions are stable for 1 week at 2-8ºC.
2. αGST Calibrators and diluted Positive Control must be used within 1 hour of preparation.
3. Once diluted, the Wash Solution can be stored at 18-25ºC for two weeks or 2-8ºC for one month.
4. Microassay wells should be stored in sealed foil pouches with desiccants at 2-8ºC until required for use. Return unused wells to storage pouch together with desiccant.

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

Note: All reagents should be allowed to reach room temperature prior to commencement of assay. Components from different batches of Argutus Medical Rat Alpha GST EIA kits must not be intermixed.

WASH SOLUTION

Prepare a 1/25 dilution of Wash Concentrate adding, for example, 10mL Wash Concentrate to 240mL deionised water as required. Prepare only the volume of Wash Solution required for the assay. Each strip of 8 microassay wells requires 35mL of Wash Solution.

CALIBRATORS

Dilute the rat αGST calibrator stock solution (5.1mg/L) as follows:

Stock:	20µL
Sample Diluent:	1000µL
Total:	1020µL @ 100µg/L (A)

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

Rat αGST Calibrator Concentration (µg/L)	Calibrator Volume (µL)	Sample Diluent Volume (µL)
100 (A)	500 (A)	0
50.0 (B)	500 (A)	500
25.0 (C)	500 (B)	500
12.5 (D)	500 (C)	500
6.25 (E)	500 (D)	500
3.12 (F)	500 (E)	500
1.56 (G)	500 (F)	500
0 (H)	0	500

POSITIVE CONTROL

Dilute Positive Control 1/50 in Sample Diluent; i.e. add 10µL Positive Control to 490µL Sample Diluent. Mix diluted Positive Control by vortexing for 5 – 10 seconds.

SAMPLE COLLECTION, HANDLING AND STORAGE

SERUM

For serum sample analysis, blood samples should be allowed to clot at room temperature for 2 hours or 2-8ºC overnight. The sample is then centrifuged (3000 rpm/10 minutes) and the serum collected.

Serum samples should be assayed immediately or may be stored at 2-8ºC for 4 days. If necessary, the samples may be frozen at -20ºC. Avoid repeated freezing and thawing. Do not store diluted samples. Samples can be stored at -20ºC for at least one month.

URINE

As soon as possible after sample collection, add 100µL of Rat Urine Stabilising Buffer to 400µL urine (4/5 dilution of sample), even if the samples are not to be stored.

Do not store samples without the addition of Rat Urine Stabilising Buffer. Rat Urine Stabilising Buffer should be added within 12 hours of sample collection.

After the addition of Rat Urine Stabilising Buffer, samples can be stored at 2-8ºC for at least 48 hours or at -20ºC for at least a month.

TISSUE CULTURE SUPERNATANT

A hepatocyte cell number of 4 x 10^5 cells /mL cell culture medium is recommended.

SAMPLE PREPARATION

SERUM

Dilute serum samples 1/50 in Sample Diluent i.e. add 10µL serum sample to 490µL Sample Diluent. Samples expected to contain levels above 2500µg/L should be diluted further.

URINE

Dilute urine samples 1/5 in Sample Diluent i.e. add 100µL urine sample to 400µL sample diluent. Samples expected to contain levels above 250µg/L should be diluted further.

TISSUE CULTURE SUPERNATANT

Samples should be diluted 1/5 to 1/30 in Sample Diluent depending on expected concentrations. These dilution factors may vary for the cell culture system used.

Do not store diluted samples of any kind.

ASSAY PROCEDURE

To obtain precise reproducible results, it is essential that care be taken during reagent addition to the microassay wells and with the washing steps. The following points should be noted:

- Do not touch the sides of the microassay wells with pipette tips during reagent addition.
- During the washing steps, 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, Positive Control and Calibrators as described in "Preparation of reagents".

1.2. Prepare samples as outlined in "Sample Preparation".

1.3. Place required number of microassay wells in the assay plate (16 for the Calibrators, two for the Positive Control plus two per sample). Add Calibrators (H-A; equivalent concentration 0-100µg/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 (20-25ºC) for 60 ± 2 minutes with uniform shaking (350 ± 10rpm).

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 fluid from 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, taking care not to touch the sides of the microassay wells with the pipette tips.

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

2.3. Wash each strip as in step 1.5 above.

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. Again, take care not to touch the sides of the microassay wells with the pipette tips.

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, the Positive Control and samples.
2. Plot a calibration curve of $A_{450/630nm}$ versus [αGST]-µg/L (4-parameter plot, refer to Figure 1).
3. Read the αGST concentration (µg/L) indicated by the mean absorbance of the Positive Control or sample from the calibration curve
4. The concentration of the Positive Control is read directly from the curve. Its value should be within the range given on the inside of the box lid.
5. Multiply the αGST concentration obtained for samples by the appropriate dilution factor.
6. Results for urine samples to which Argutus Medical Rat Urine Stabilising Buffer has been added should be multiplied by a factor of 1.25 to allow for 4/5 dilution of the sample.
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.
8. Please see Appendix 1 for instructions on how to express urinary αGST as rate (ng/min).

EXAMPLE OF A CALIBRATION CURVE

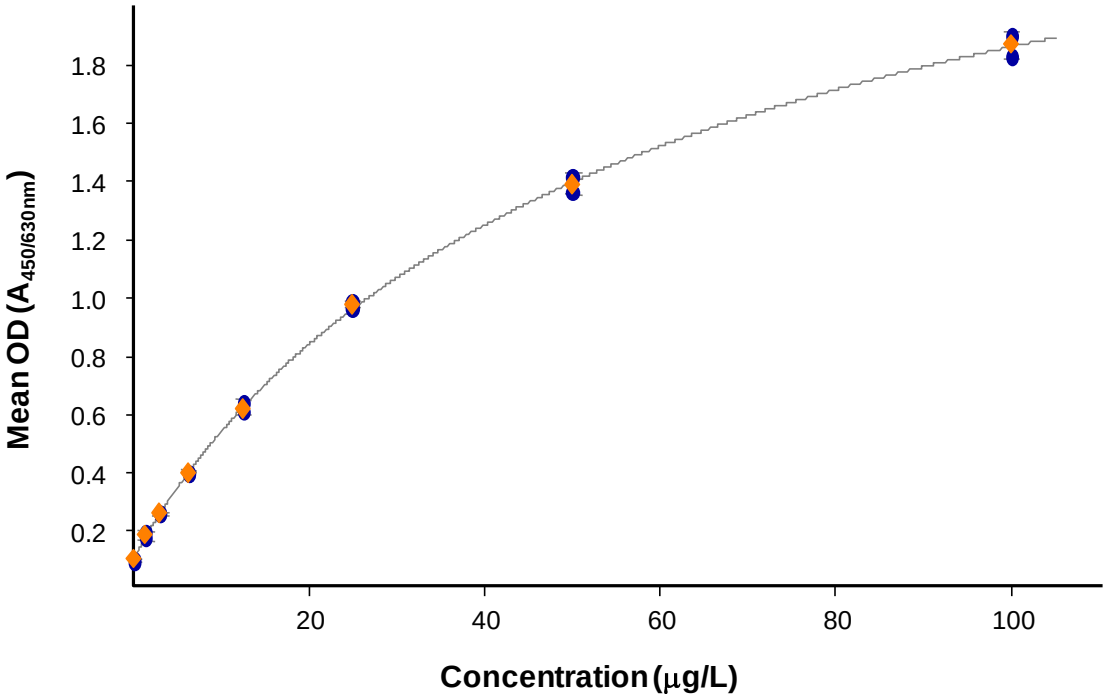

Figure 1: Typical calibration curve obtained using Argutus Medical Rat Alpha GST EIA. 4-parameter plot of $A_{450/630nm}$ versus [αGST] µg/L. Assay range is 1.56 – 100µg/L αGST.

PERFORMANCE CHARACTERISTICS

REFERENCE RANGES

SERUM

Sprague-Dawley Rats 43 ± 56µg/L (mean ± 2SD)

URINE

Sprague-Dawley Rats 46.9 ± 44.6µg/L (mean ± 2SD)

Wistar Rats 23.0 ± 26.4µg/L (mean ± 2SD)

Normal ranges may vary between different rat strains. Therefore, it is important for each laboratory to assess a normal range for the rat strain used in the study. Contact Argutus Medical for advice.

MEASURING RANGE

The calibration range covers 1.56-100 µg/L corresponding to 7.8 to 5000µg/L using 1/50 dilution (serum) or 9.75 to 625 µg/L in stabilised urine samples diluted 1/5. This range may be extended by increasing sample dilution.

REPRODUCIBILITY

Table 1. Inter-assay Variation of the Argutus Medical Rat Alpha GST EIA

Sample	Mean Final Conc. αGST µg/L	% CV	n
Low Urine	45	8.8	11
High Urine	247	12.0	11
Low Serum	1545	10.9	11
High Serum	3472	13.5	11

LIMIT OF DETECTION

The detection limit of Argutus Medical Rat Alpha GST EIA is 0.2µg/L in the microassay well, equivalent to 10µg/L in a serum sample diluted to 1/50, or 1.25µg/L in a stabilised urine sample, diluted 1/5.

SPECIFICITY

The Argutus Medical Rat Alpha GST EIA is highly specific for rat αGST (YaYc isotypes). No significant cross-reactivity is observed with Yp or Yb1 isoforms.
Cross reactivity with human, canine and porcine αGST is undetectable.

LINEARITY

Table 2. Linearity testing using Argutus Medical Rat Alpha GST EIA

Sample	Test Dilution	Expected Final Conc. αGST µg/L	Measured Final Conc. αGST µg/L	% Recovery
Low Urine	5	N/A	44	N/A
	10	44	42	95%
	20	44	40	91%
	40	44	44	100%
High Urine	5	N/A	220	N/A
	10	220	205	93%
	20	220	210	96%
	40	220	252	115%
Low Serum	25	N/A	1520	N/A
	50	1520	1445	95%
	100	1520	1390	91%
	200	1520	1500	99%
High Serum	50	N/A	3035	N/A
	100	3035	2940	97%
	200	3035	2400	79%
	400	3035	2600	86%

SPIKE RECOVERY

Table 3. Spike Recovery testing using Argutus Medical Rat Alpha GST EIA

Sample	αGST Spike Level	Measured Final Conc. αGST µg/L	Expected Final Conc. αGST µg/L	% Recovery
Low Urine	Low	152	154	99%
	High	536	574	93%
High Urine	Low	298	278	107%
	High	722	698	103%
Low Serum	Low	1455	1627	89%
	High	2100	2047	103%
High Serum	Low	2660	3002	89%
	High	3250	3422	95%

APPENDIX 1

EXPRESSING THE RELEASE OF αGST IN TERMS OF RATE

In situation of unusual dieresis, e.g., poly- or oligouria, it may be more relevant to express αGST release in terms of rate (αGST ng/min) rather than concentration. The rate of release is obtained as follows:

URINE COLLECTION

Collect urine samples as described in "Sample Collection and Handling".
Note the period of urine collection (T) in minutes and total urine volume (V).

CALCULATION OF αGST RELEASE RATE

1. Determine urinary αGST levels using the Argutus Medical Rat Alpha GST EIA (µg/L).
2. Note the period over which the urine was collected (T) in minutes.
3. Note the urine volume in mL (V).
4. Calculate the excretion rate as follows:

$$\text{αGST (ng/min)} = \frac{([\text{αGST}]\ \mu\text{g/L}) \times V}{T}$$

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: **100µL standards/positive control/sample**
   → Incubate: **60 min**
   → Wash: **6 X 300µL**

2. Pipette: **100µL enzyme conjugate**
   → Incubate: **60 min**
   → Wash: **6 X 300µL**

3. Pipette: **100µL substrate**
   → Incubate: **15 min**

4. Pipette: **100µL stop solution**
   → Read immediately: **450nm/630nm**

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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
BIO91	Human Alpha GST EIA	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

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