

**REF** **BIO64RAT**  
**96 well plate**



**ARGUTUS MEDICAL**

# **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 ( $\alpha$ GST) in biological fluids such as serum, urine and tissue culture supernatants.

## **BACKGROUND**

### **HEPATIC STUDIES**

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

### **RENAL STUDIES**

In the rat kidney,  $\alpha$ GST is found in the proximal convoluted tubules<sup>10</sup>. It is readily and rapidly released into the urine when renal tubular injury occurs<sup>11-13</sup>. Urinary  $\alpha$ GST levels correlate closely with the time course<sup>11</sup> and the severity of renal injury<sup>12</sup>. Urinary  $\alpha$ GST levels are more sensitive indicators of renal tubular injury than serum creatinine<sup>12</sup>. The assay can be used together with Argutus Medical's Rat GST Yb1 EIA and RPA-1 EIA, which monitor injury to the distal tubules and collecting ducts respectively, to provide a broad picture of injury to the renal tubules.

## **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  $\alpha$ GST IgG. The resultant colour intensity is proportional to the amount of  $\alpha$ GST in the sample. The assay range is 1.56-100 $\mu$ g/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: 

PLA
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96 wells (12x8 break well strips) coated with IgG directed against rat  $\alpha$ GST.  
READY TO USE
  
2. Enzyme Conjugate: 

CONJ
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Anti-rat  $\alpha$ GST IgG conjugated to horseradish peroxidase (12mL).  
Contains Thiomersal.  
READY TO USE
  
3.  $\alpha$ GST Calibrator Concentrate: 

CAL
-----

  
Purified rat  $\alpha$ GST (YaYc isoform) with added stabilisers (200 $\mu$ L).  
Contains Thiomersal and sodium azide  
**Store at -20°C until required**  
STOCK SOLUTION
  
4. Positive Control: 

CONTROL	+
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Purified rat  $\alpha$ GST with added stabilisers (500 $\mu$ L).  
Contains Thiomersal and sodium azide  
**Store at -20°C until required**  
STOCK SOLUTION
  
5. Rat Urine Stabilising Buffer (10mL) 

BUF	NEPH
-----	------

  
Contains Thiomersal and sodium azide  
READY TO USE
  
6. Sample Diluent: (55mL). 

DIL	SPE	1X
-----	-----	----

  
Contains sodium azide  
READY TO USE
  
7. Wash Concentrate: 

BUF	WASH	25X
-----	------	-----

  
25X Tris buffered saline / Tween-20 (TBST, 55mL)  
Contains Thiomersal  
CONCENTRATE
  
8. Substrate: 

SUBS	TMB
------	-----

  
Stabilised TMB solution (11mL).  
READY TO USE
  
9. Stop Solution: 1N 

SOLN	STP
------	-----

  
Sulphuric Acid (11mL).  
READY TO USE
  
10. Instructions for use 

INS
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## **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.
- Some reagents contain Thiomersal, which may be toxic if ingested.
- Some reagents contain sodium azide which may form potentially explosive metal azides with lead and copper plumbing. For disposal, reagent should be flushed with large volumes of water to prevent azide build up.
- 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.

**WARNING:** This product contains a chemical known to the State of California to cause birth defects or other reproductive harm (California Prop 65: Thiomersal).

### **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 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 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.

## **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 -20°C 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 2-8°C.
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 18-25°C for 2 weeks or 2-8°C for 2 months.
4. Microassay wells should be stored in sealed foil pouches with desiccants at 2-8°C until required for use. Return unused wells to 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. 20mL pipette
4. Pipette pump
5. 500mL beaker
6. 500mL graduated cylinder
7. Test tubes (~1.5mL)
8. Timer
9. Deionised/distilled water
10. Plate shaker
11. ELISA plate reader capable of reading at 450nm (using 630nm as reference if possible)

## **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 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  $\alpha$ GST Calibrator stock solution (5.05mg/L) thus:

Stock: 20 $\mu$ L  
Sample Diluent: 1000 $\mu$ L  
Total: 1020 $\mu$ L @ 100 $\mu$ g/L  
**(SOLUTION A)**

Using labelled test tubes, prepare calibrators as follows:

<b>To Prepare Calibrator (ug/L)</b>	<b>Volumes of Solutions Required</b>
100 (A)	Solution A
50.0 (B)	Add 500 $\mu$ L of A to 500 $\mu$ L of Sample Diluent
25.0 (C)	Add 500 $\mu$ L of B to 500 $\mu$ L of Sample Diluent
12.5 (D)	Add 500 $\mu$ L of C to 500 $\mu$ L of Sample Diluent
6.25 (E)	Add 500 $\mu$ L of D to 500 $\mu$ L of Sample Diluent
3.12 (F)	Add 500 $\mu$ L of E to 500 $\mu$ L of Sample Diluent
1.56 (G)	Add 500 $\mu$ L of F to 500 $\mu$ L of Sample Diluent
0 (H)	Sample Diluent

### **POSITIVE CONTROL**

Dilute Positive Control 1/50 in Sample Diluent; i.e. add 10 $\mu$ L Positive Control to 490 $\mu$ L Sample Diluent.

## **SAMPLE COLLECTION, HANDLING AND STORAGE**

### **SERUM**

For serum sample analysis, blood samples should be allowed to clot at room temperature for 2 hours or 2-8°C 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 2-8°C for 4 days. If necessary, the samples may be frozen at -20°C. Avoid repeated freezing and thawing. Do not store diluted samples. Samples can be stored at -20°C for at least 1 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 2-8°C for at least 48 hours or at -20°C for at least a month.

### **TISSUE CULTURE SUPERNATANT**

A hepatocyte cell number of  $4 \times 10^5$  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 with the washing steps. The following points should be noted:

- 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; 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 (20-25°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 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

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 1.5 above.

### **3. COLOUR DEVELOPMENT**

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

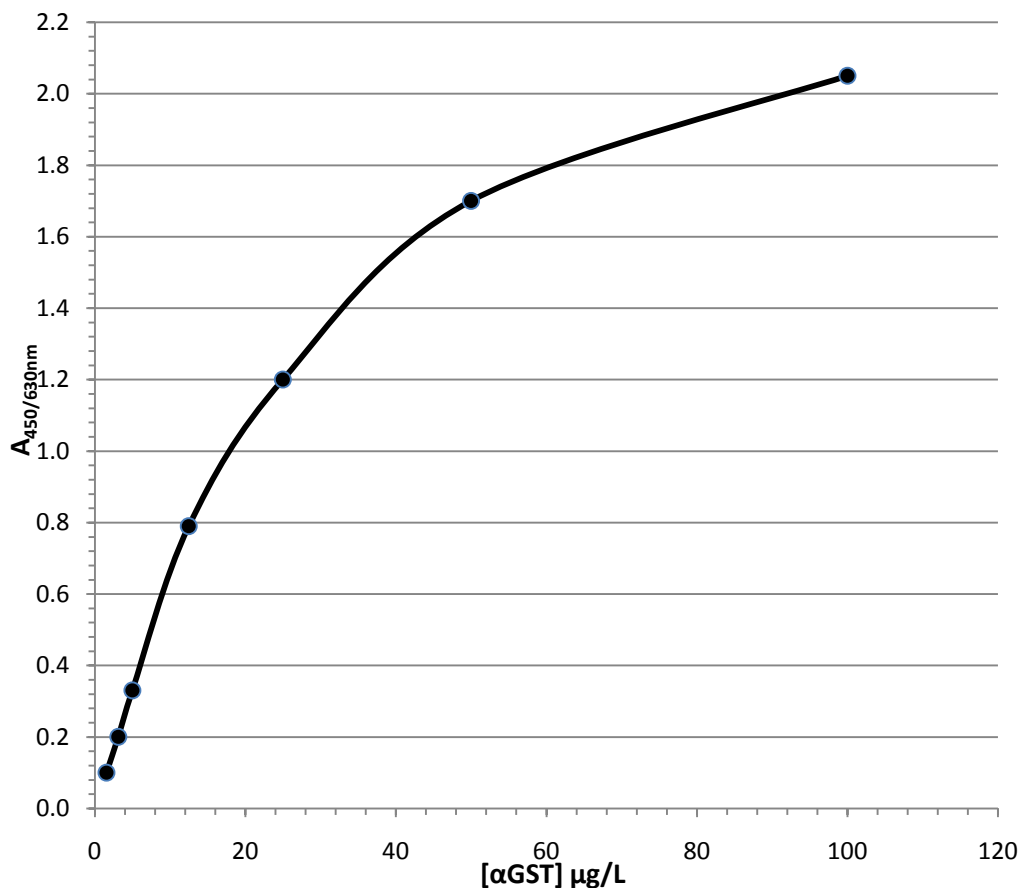
#### 4. STOP

- 4.1. Stop the reaction by addition of **100 $\mu$ L** 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  $[\alpha\text{GST}]-\mu\text{g/L}$  (See Figure 1).
3. Read the  $\alpha\text{GST}$  concentration ( $\mu\text{g/L}$ ) 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  $\alpha\text{GST}$  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 Alpha GST as rate (ng/min).

## **EXAMPLE OF A CALIBRATION CURVE**



**Figure 1:** Typical calibration curve obtained using Argutus Medical Rat Alpha GST EIA. Plot of A<sub>450/630nm</sub> versus [αGST] μg/L.

## **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 5000µ/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. *Intra-assay*** Variation of the Argutus Medical Rat Alpha GST EIA

Sample	Mean (µg/L)	%CV	n
Urine low	34.7	4.1	20
Urine medium	86.0	4.3	20
Urine high	184	5.6	20
Serum low	186	3.1	20
Serum medium	410	2.6	20
Serum medium/high	916	4.7	20
Serum high	1201	6.0	20

**Table 2. *Inter-assay*** Variation of the Argutus Medical Rat Alpha GST EIA

Sample	Mean (µg/L)	%CV	n
Urine low	33.1	7.1	10
Urine medium	78.6	4.6	10
Urine high	178	6.4	10
Serum low	176	6.2	10
Serum medium	398	7.2	10
Serum medium/high	864	4.9	10
Serum high	1192	5.6	10

## 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 isoforms). No significant cross-reactivity is observed with Yp or Yb1 isoforms. Cross reactivity with human, canine and porcine αGST is undetectable.

## LINEARITY

### Serum

Dilution Factor	Sample 1 (µg/L)	Sample 2 (µg/L)
1/40	166	1040
1/80	181	1160
1/160	179	1208
1/320	187	1187

### Urine

Dilution Factor	Sample 1 (µg/L)	Sample 2 (µg/L)
1/4	31.2	185
1/8	35.7	186
1/16	47.0	193

## APPENDIX 1

### EXPRESSING THE RELEASE OF αGST IN TERMS OF RATE

In situation of unusual diuresis, 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 GST Yb1 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:

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

## 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: **100 $\mu$ L standards/positive control/sample**



Incubate: **60 min**



Wash: **6 X 300 $\mu$ L**

2. Pipette: **100 $\mu$ L enzyme conjugate**



Incubate: **60 min**



Wash: **6 X 300 $\mu$ L**

3. Pipette: **100 $\mu$ L substrate**



Incubate: **15 min**

4. Pipette: **100 $\mu$ L stop solution**



Read immediately: **450nm/630nm**

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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>BIO76YB1</b>	Rat Yb1 GST EIA	GSTYb1 ( $\mu$ GST) 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	$\alpha$ GST in human urine
<b>BIO60HEPA</b>	HEPKIT® Alpha GST EIA	$\alpha$ GST in human serum and plasma
<b>BIO60HEPAS</b>	High Sensitivity Alpha GST EIA	$\alpha$ GST in human serum, plasma and tissue
<b>BIO85</b>	Pi GST EIA	$\pi$ GST in human urine and plasma
<b>BIO83</b>	Urinary Collagen IV EIA	Collagen IV in human urine
<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



**ARGUTUS MEDICAL**

**Argutus Medical Ltd.**  
**Unit 9 Trinity Technology & Enterprise Campus,**  
**Pearse Street,**  
**Dublin 2,**  
**Ireland**  
**Tel: +353 1 670 8576**  
**Fax: +353 1 670 8575**  
[info@argutusmed.com](mailto:info@argutusmed.com)  
<http://www.argutusmed.com>

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