REF BIO91RUO
96 well plate

Human Alpha GST EIA

Enzyme Immunoassay

Instructions for Use

For Research Use Only

Not for use in Diagnostic Procedures

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INTENDED USE

The EKF Diagnostics Alpha GST EIA provides a method for the quantitative determination of alpha glutathione S-transferase (αGST) in human urine, serum and plasma. To assay αGST in other media or assay other GST subclasses, please contact EKF Diagnostics for further information. The EKF Diagnostics Alpha GST EIA is for research use only and not for use in diagnostic procedures.

BACKGROUND

URINE STUDIES

In kidney, alpha glutathione S-transferase (αGST) is found in the proximal tubule region whereas Pi glutathione S-transferase (πGST) is confined mainly to the distal tubules[1]. Low levels of αGST are released into the urine in normal individuals, as confirmed by immunoassay and Western blot anlaysis[2]. Any event which precipitates proximal tubular damage may cause increased release of αGST into urine and elevations of urinary αGST levels have been shown to be indicative of proximal tubule damage in nephrotoxicity[3-5], environmental toxicity[6], surgery[7], acute renal failure[8] and transplantation[9-12]. The release of πGST has been shown to be associated with distal tubular damage[6], thus simultaneous measurement of αGST and πGST may allow discrimination between proximal and tubular damage[5, 9-11].

SERUM STUDIES

In liver, alpha glutathione S-transferase is located in the hepatocytes whereas pi GST (αGST) is confined to the intrahepatic bile duct cells[1, 13-14]. This heterogeneous GST subclass distribution suggests that the isoenzymes have unique *in vivo* functions in different hepatic regions and that the detection of GST subclass levels in biological fluids would be of significant use in monitoring the integrity of specific hepatic regions. Currently, liver injury is studied by the measurement of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A disadvantage of these markers is that they are not distributed uniformly throughout the liver, the periportal concentration being greater than the centrilobular[15]. In contrast, αGST has been found to be equally distributed in both the centrilobular and periportal regions[13-14]. Since the centrilobular hepatocytes are very susceptible to damage in a variety of conditions including Allograft Rejection[16-18], Viral Hepatitis[19], and Hepatotoxicity[20,] αGST is a more sensitive indicator of hepatic status.

EKF Diagnostics Alpha GST EIA is a specific, precise immunoassay for αGST[21,22] and, being a quantitative test, is unaffected by modulators of enzyme activity (e.g. bile salts and bilirubin)[21]. Thus, it is now possible to use αGST quantitation to study the hepatocellular status of individuals at risk of hepatic damage.

ASSAY PRINCIPLE

EKF Diagnostics Alpha 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-αGST IgG. The resultant colour intensity is proportional to the amount of αGST present in the sample. The assay range is 2.5 – 80 µg/L.

COMPONENTS

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

   PLA

2. Calibrator, 0.2mL (2mg/L)
   Purified αGST in stabilising diluent containing ProClin 950 and Bronidox L as preservatives.
   25X CONCENTRATE

CAL

DIL	SPE	1X

BUF	WASH	25X

CONTROL	+

CONJ	EN	1X

SUBS	TMB

SOLN	STOP

BUF	USB

INS

PRECAUTIONS

SAFETY

- EKF Diagnostics Alpha GST EIA is for research use only and not for use in diagnostic procedures.

- EKF Diagnostics Alpha 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 of 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 components containing ProClin 950 are classified as per applicable European Community (EC) Directives as: Irritant (Xi). The following are the 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 beyond 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 Buffer. 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 wash step.
- 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 2-8°C until required for use. Return unused wells to the storage pouch together with desiccants.
3. Alpha GST Calibrators must be used within 30 minutes of preparation.
4. Prepared Wash Buffer (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 shaker
10. Room temperature incubator
11. Vortex

PREPARATION OF REAGENTS

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

2. CALIBRATORS

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

Stock:	40 µL
Sample Diluent:	960 µL
Total:	1000 µL @ 80µg/L (A)

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

αGST Calibrator Concentration (µg/L)	Calibrator Volume (µL)	Sample Diluent Volume (µL)
80 (A)	300 (A)	0
40 (B)	300 (A)	300
20 (C)	300 (B)	300

10 (D)	300 (C)	300
5 (E)	300 (D)	300
2.5 (F)	300 (E)	300
0 (G)	0	300

SAMPLE COLLECTION

URINE

EKF Diagnostics Alpha GST EIA can be used to measure αGST in any urine sample but, due to the diurnal variation in proteinuria[23], it is important for optimal results that timed, quantitative, urine samples are collected and the collection period and volume recorded. This will enable αGST 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 EKF Diagnostics for advice.

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

SERUM / PLASMA

EKF Diagnostics Alpha GST EIA can be used to measure αGST in serum, EDTA or sodium-heparin plasma samples. Collect all blood samples in an appropriate tube and observe routine precautions for venipuncture. Mix the tube immediately after collection by inverting several times. Centrifuge within 3 hours from time of collection and transfer the sample from the original tube for storage at 2-8°C. If not tested within 24 hours, aliquot the sample and store at -20°C or -80°C. Inspect samples for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

SAMPLE HANDLING AND STORAGE

URINE

Do not store urine samples without the addition of Urine 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 of samples should be avoided to prevent loss of αGST (up to 20% drop in αGST concentration observed after 3 freeze-thaw cycles as measured by EIA).

SERUM / PLASMA

Serum and plasma 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 of samples should be avoided to prevent loss of αGST (up to 20% drop in αGST concentration observed after 3 freeze-thaw cycles as measured by EIA).

SAMPLE PREPARATION

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

SERUM / PLASMA
Immediately prior to the assay, dilute samples dilute 1/5 by adding 50µL sample to 200µL Sample Diluent.

NOTE: If multiple sample additions (>10 duplicate samples) are to be undertaken then, to facilitate transfer to the assay plate, samples can 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 for each of the controls and samples). Add Calibrators (G-A; equivalent concentration 0 - 80µg/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 Buffer (250µL - 350µL/well). When complete, firmly tap the plate against a paper towel to ensure complete removal of Wash Buffer 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.

   2.2. Again cover the microassay plate and incubate at room temperature (20-25°C) for 30 ± 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 adding 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 sample.
2. Plot a calibration curve of $A_{450/630nm}$ versus [αGST] (µg/L) (4-parameter plot, Figure 1).
3. Read the [αGST] (µg/L) indicated by the mean absorbances of the samples from the calibration curve.
4. Multiply the calculated [αGST] by the appropriate dilution factor in order to obtain the actual [αGST]. Results for stabilised urine samples should be multiplied by an additional factor of 1.25 to compensate for the dilution of sample with Urine 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 specified 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.

REFERENCE RANGES

Samples were obtained from apparently healthy donors without any clinical abnormal indications. αGST levels were determined using the EKF Diagnostics Alpha GST EIA in order to establish the αGST concentration in the normal population.

The reference interval (5^{th} to 95^{th} percentiles) for EKF Diagnostics Alpha GST EIA is 0-29.0µg/L in urine (n=120). The reference interval (5^{th} to 95^{th} percentiles) for EKF Diagnostics Alpha GST EIA is 0-12.0µg/L in serum (n=120). The reference intervals reflect 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 is 2.5 - 80µg/L, which corresponds to 6.25 - 200µg/L in stabilised urine samples diluted 1/2 in Sample Diluent or 12.5 - 400µg/L in serum/plasma samples diluted 1/5 in Sample Diluent. This range may be extended by increasing sample dilution.

PRECISION

A 10-day precision study was performed on the EKF Diagnostics Alpha 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 EKF Diagnostics Alpha GST EIA and 4 different operators. Three urine pools containing endogenous αGST and four control samples spiked with αGST were assayed in duplicate at two separate times per day for 10 days. The data is summarized in the following table:

Sample	n	Mean [αGST] µg/L	Repeatability		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Urine Pool	40	**24.0**	0.52	2.2	2.05	8.5	0.87	3.6	2.28	9.5
Medium Urine Pool	40	**43.8**	1.10	2.5	2.51	5.7	1.23	2.8	3.01	6.9
High Urine Pool	40	**195.4**	5.06	2.6	11.84	6.1	12.64	6.5	18.05	9.2
High Plasma Control	40	**1581.6**	86.05	5.4	89.91	5.7	232.14	14.7	263.39	16.7
Low Urine Control	40	**15.3**	0.36	2.4	1.00	6.6	0.00	0.0	1.07	7.0
Medium Urine Control	40	**41.5**	0.48	1.2	2.46	5.9	0.22	0.5	2.51	6.1
High Urine Control	40	**75.0**	1.72	2.3	4.38	5.8	0.00	0.0	4.71	6.3

SPECIFICITY

EKF Diagnostics Alpha GST EIA is highly specific for αGST. No cross-reactivity was observed with µGST at 500µg/L, or πGST at 500µg/L.

SENSITIVITY

The limit of detection (LoD) of EKF Diagnostics Alpha 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 1.9µg/L αGST, which corresponds to 4.75µg/L in a stabilised urine sample diluted 1/2 or 9.5µg/L in a serum/plasma sample diluted 1/5.

LINEARITY UPON DILUTION

Sample pools with αGST concentrations ranging from 14.7µg/L to 15400µg/L were serially diluted with EKF Diagnostics Alpha GST EIA Sample Diluent and assayed. % Recovery of αGST recovery was calculated as (Measured [αGST] µg/L / Expected [αGST] µg/L) x 100. Recovery of αGST was found to be 100±10% (serum 92-105%, EDTA plasma 94-105%, heparin plasma 94-105%, and stabilised urine 91-107%).

INTERFERENCE

Potentially interfering endogenous substances were evaluated to determine their effect on αGST recovery using EKF Diagnostics Alpha GST EIA. The endogenous substances listed below were spiked into urine and serum pools containing endogenous αGST at a concentration of ~300µg/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:

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

Interfering Substance	Interferent Concentration (mg/dL)	Interference in Urine (% Bias)	Interference in Serum (% Bias)
Bilirubin (conjugated)	20	0%	-4%
Bilirubin (unconjugated)	20	1%	2%
Haemoglobin	2000	-7%	-1%
Albumin	6000	3%	1%
Lipid*	1500	-5%	1%
Human IgG	4	3%	3%
Tamm-Horsfall Protein**	5	-22%	-

* *Performed with 20% intralipid.*

** *The endogenous concentration of the urine sample pool used for testing was unknown. The average THP concentration in healthy subjects is estimated at 6.1 – 9.0 mg/dL[24] and thus, the final concentration is likely to be in excess of 11.1mg/dL.*

No significant interference was observed in this assay with EDTA up to 3.4µmol/L or sodium heparin up to 3,000U/L. Studies also indicated that samples with rheumatoid factor do not cause interference.

EXAMPLE OF A CALIBRATION CURVE

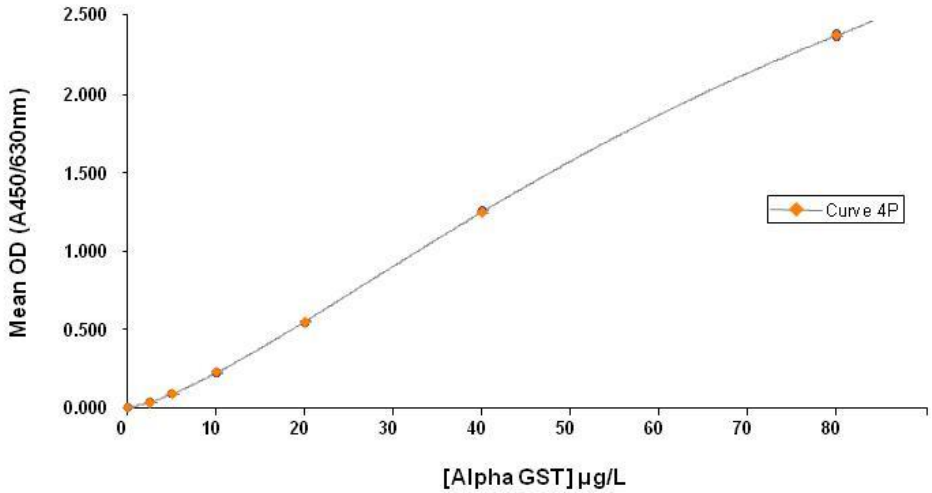

Figure 1. Typical calibration curve obtained using EKF Diagnostics Alpha GST EIA.
4-parameter plot of $A_{450/630nm}$ versus [αGST] µg/L. Assay range is 2.5 – 80 µg/L αGST.

WARRANTY

The performance data presented here was obtained using the procedure described. Any change or modification of the procedure, not recommended by EKF Diagnostics, may affect the results, in which case EKF Diagnostics disclaims all warranties, expressed, implied or statutory, including implied merchantability and fitness for use. In the case of such an event, EKF Diagnostics shall not be liable for damages, direct or consequential.

APPENDIX 1

EXPRESSING αGST RELEASE RATE

Excretion 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'. Note the time of urination (T2), time of the previous urination (T1) and the total urine volume (V).

CALCULATION OF αGST EXCRETION RATE

1. Determine urinary αGST levels (µg/L) using EKF Diagnostics Alpha GST EIA.
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 Buffer and Calibrators.
   1.2. Prepare Samples
   1.3. Place microtitre wells in the assay plate. Add Calibrators, Positive Control and diluted samples **(100µL/well)**, in duplicate, to the microtitre plate.
   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 Buffer **(250µL-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 30 ± 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 adding **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. **CALCULATE RESULTS**

INTERPRETATION OF SYMBOLS

Positive Control Range	
Batch code	
Catalogue Number	
Temperature limitation	
Use by end of	
Manufacturer	
Biohazardous	

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