

Catalogue No:

BIO76YB1

Kit Format:

96 Wells

# **Biotrin GSTYb1 EIA -Rat GST-Mu**

## **Enzyme Immunoassay for GSTYb1**

Instructions for Use



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## **INTENDED USE:**

The Biotrin GSTYb1 EIA provides a method for the quantitative determination of rat Mu glutathione S-transferase ( $\mu$ GST, GSTYb1). For the assay of GSTYb1 in other species and other GST subclasses, contact Biotrin for advice.

## **BACKGROUND:**

GSTYb1 is found in high concentrations in the distal straight and convoluted regions of rat renal tubules, whereas alpha GST ( $\alpha$ GST, YaYc) is found mainly in the proximal tubules<sup>1</sup>. GSTYb1 is found in the urine of normal rats as confirmed by enzyme immunoassay<sup>2-3</sup>. Any event which precipitates distal tubule damage may cause the release of GST leading to an increase in urinary levels<sup>4-7</sup>. Thus, an elevation in urinary GSTYb1 levels may be indicative of distal tubule damage<sup>6</sup>.


The assay can be used together with Biotrin's Rat Alpha GST EIA and RPA-1 EIA, which monitor injury to the proximal tubules and collecting ducts respectively, to provide a broad picture of injury to the renal tubules.

## **ASSAY PRINCIPLE:**

The Biotrin GST Yb1 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-GSTYb1 IgG. The resultant colour intensity is proportional to the amount of GSTYb1 present in the sample. The assay range is 0 - 100  $\mu$ g/L.

## **COMPONENTS:**

1. Antibody Coated Microassay Plate  
12 x 8 well strips coated with IgG  
directed against GSTYb1. Breakapart wells.  
READY TO USE PLA
2. GSTYb1 Calibrator  
Purified GSTYb1 in 50% (v/v) glycerol  
(5mg/L, 100 $\mu$ L).  
Contains thiomersal and sodium azide.  
STOCK SOLUTION CAL
3. Sample Diluent DIL SPE 1X  
Protein containing solution with added  
stabilisers (50mL). Contains thiomersal and sodium azide.  
READY TO USE
4. Wash Concentrate BUF WASH 25X  
25x tris buffered saline/Tween-20  
(TBST, 55mL). Contains thiomersal.  
CONCENTRATE

- |     |  |   |      |    |
|-----|--|---|------|----|
| 5.  | Positive Control<br>GSTYb1 in protein containing solution with stabilisers (4.5mL).<br>Contains thiomersal and sodium azide.<br>READY TO USE | PC  | +    |    |
| 6.  | Conjugate<br>Anti-rat GSTYb1 IgG conjugated to horseradish peroxidase (12mL).<br>Contains Thiomersal.<br>READY TO USE                        | CONJ  | ENZ  | 1X |
| 7.  | Substrate<br>Stabilised liquid TMB solution (11mL).<br>READY TO USE  | SUBS  | TMB  |    |
| 8.  | Stop Solution<br>1N Sulphuric Acid (11mL).<br>READY TO USE   | SOLN  | STP  |    |
| 9.  | Rat Urine Stabilising Buffer (10mL).<br>Contains thiomersal and sodium azide<br>READY TO USE   | BUF   | NEPH |    |
| 10. | Product Insert   |  |      |    |

## **PRECAUTIONS:**

### **SAFETY**

- The Biotrin GSTYb1 EIA kit is for *in-vitro* diagnostic use only.
- The Biotrin GST Yb1 EIA kit is intended for use by qualified laboratory staff only.
- 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 that 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, 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, 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.
- **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 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.
- 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.
- Always keep the upper surface of the wells free of droplets. Drops should be gently blotted dry on completion of the procedural 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 plate wells should be stored in sealed bags with desiccants at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.
3. Calibrators must be used within 30 minutes of preparation.
4. Once diluted, the Wash Solution can be stored at 18-25°C for 2 weeks or 2-8°C for 1 month.

## **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. 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:**

**NOTE:** All reagents should be allowed to reach room temperature prior to commencement of assay.

### **1. WASH SOLUTION (TBST)**

Perform a 1/25 dilution of Wash Concentrate adding, for example, 10mL of Wash Concentrate to 240mL 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 the 100µg/L Calibrator (A) from the Calibrator stock solution as follows:

$$\begin{array}{r} \text{Stock: } 20\mu\text{L} \\ \text{Sample Diluent: } 980\mu\text{L} \\ \hline \text{Total: } 1000\mu\text{L} \quad @ \quad 100\mu\text{g/L (A).} \end{array}$$

Using labelled test tubes, prepare further calibrators as follows:

To prepare calibrator: (GSTYb1 $\mu\text{g/L}$ )	Volumes of solutions required
100 (A)	Solution A
50 (B)	Add 500 $\mu\text{L}$ of A to 500 $\mu\text{L}$ of Sample Diluent
25 (C)	Add 500 $\mu\text{L}$ of B to 500 $\mu\text{L}$ of Sample Diluent
12.5 (D)	Add 500 $\mu\text{L}$ of C to 500 $\mu\text{L}$ of Sample Diluent
6.25 (E)	Add 500 $\mu\text{L}$ of D to 500 $\mu\text{L}$ of Sample Diluent
3.13 (F)	Add 500 $\mu\text{L}$ of E to 500 $\mu\text{L}$ of Sample Diluent
1.56 (G)	Add 500 $\mu\text{L}$ of F to 500 $\mu\text{L}$ of Sample Diluent
0 (H)	Sample Diluent

Standards must be used before stated 30min see page 4.

### **SPECIMEN COLLECTION, HANDLING AND STORAGE:**

The Biotrin Rat GSTYb1 EIA can be used to measure GSTYb1 in any urine sample, but it is recommended for optimal results that timed, quantitative, urine samples are used. This will enable GSTYb1 release to be expressed as rate (ng/min); see appendix 1). It is recommended that urine samples are collected at the same time of day and for the same time period on every occasion. Contact Biotrin International for advice.

As soon as possible after sample collection, add 200 $\mu\text{L}$  of Rat Urine Stabilising Buffer to 800 $\mu\text{L}$  urine (4/5 dilution of sample), even if the samples are not to be stored. The same stabilised urine sample can be used for the assay of  $\alpha\text{GST}$  and RPA-1 using the respective Biotrin assays.

In the absence of bacterial growth, no change in GSTYb1 levels is observed in rat urine which has been stored at 2-8°C for up to 14 days. In the presence of Rat Urine Stabilising Buffer, samples can be stored at 2-8°C for 14 days or at -20°C for at least 1 month.

### **SAMPLE PREPARATION:**

Immediately prior to the assay, dilute samples 1/10 by adding 50 $\mu\text{L}$  sample to 450 $\mu\text{L}$  Sample Diluent. If multiple sample addition (>10 duplicate samples) is 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: 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 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 (16 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 (**H-A; 0-100 $\mu$ g/L**), Positive Control and diluted samples(**100 $\mu$ L/well**), in duplicate, to the Microassay plate.

1.4 Cover the Microassay plate and incubate at room temperature (20-25°C) for **60  $\pm$  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 4 times with Wash Solution (**250 $\mu$ L-350 $\mu$ L/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 **100 $\mu$ L** Conjugate / well.

2.2 Cover the Microassay plate and incubate at room temperature (20-25°C) for **60  $\pm$  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 Step 1.5 .

## 3. COLOUR DEVELOPMENT

3.1 Add **100 $\mu$ L** Substrate / well using a multichannel pipette 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 at 450nm using 630nm as reference (if available).

## CALCULATION OF RESULTS:

1. Calculate the mean absorbance for each Calibrator and Sample.
2. Plot a calibration curve of  $A_{450/630nm}$  versus [GSTYb1] ( $\mu$ g/L) (Lin-log plot). (See Figure 1).
3. Read the [GSTYb1]( $\mu$ g/L) indicated by the mean absorbances of the samples from the Calibration curve.
4. Multiply the calculated [GSTYb1] by the appropriate dilution factor in order to obtain the actual [GSTYb1].
5. The concentration for the Positive Control is read directly from the curve. Its value should be within the range given on the inside of the box lid.
6. Results for samples to which Biotrin Rat Urine Stabilising Buffer has been added should be further multiplied by a factor of 1.25 to allow for the 4/5 dilution of the sample.

7. Concentration of samples with readings outside the curve are invalid and must be repeated with a higher dilution factor. It is not acceptable to extrapolate data.

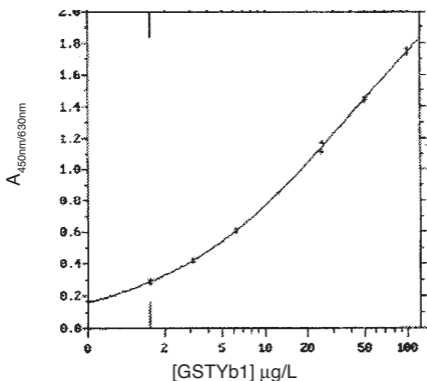


Figure 1: Typical Calibration curve obtained using the Biotrin GSTYb1 EIA. Lin-log plot of  $A_{450nm/630nm}$  versus  $[GSTYb1] \mu g/L$ .

## **PERFORMANCE CHARACTERISTICS:**

### **NORMAL RANGE**

Normal ranges may vary between different strains of rat. The concentration of GSTYb1 in urine from Sprague Dawley rats (n=31) was  $33.9 \pm 14.6 \mu g/L$  ( $\bar{x} \pm S.D.$ ). In Wistar rats (n=20), the GSTYb1 concentration was  $11.4 \pm 5.3 \mu g/L$  ( $\bar{x} \pm S.D.$ ). However, each individual laboratory should establish its own normal range.

### **MEASURING RANGE**

The calibration curve range covers the range 1.56 to  $100 \mu g/L$ , corresponding to 19.5 -  $1250 \mu g/L$  GSTYb1 in stabilised urine samples diluted 1/10. This range may be extended by increasing sample dilution.

### **SPECIFICITY**

The Biotrin GSTYb1 EIA is highly specific for the detection of GSTYb1 ( $\mu GST$ ). No significant cross-reactivity is observed with either alpha (YaYc) or pi (Yp) isoforms of rat GST.

## **SAMPLE RECOVERY**

Recovery of added GSTYb1 was 97% over the range 30 - 100µg/L.

## **LIMIT OF DETECTION**

The sample detection limit of Biotrin GSTYb1 EIA kit is 0.2µg/L: equivalent to 2.5µg/L in a stabilised urine sample diluted 1/10.

## **REPRODUCIBILITY**

Sample	Mean µg/L	C.V.%	n
1	8.3	5.1	10
2	31.4	6.6	10
3	51.2	7.1	10

Table 1. Intra-assay variation of the Biotrin GST Yb1 EIA.

Sample	Mean µg/L	C.V.%	n
1	7.4	8.8	10
2	25.1	9.4	10
3	56.5	8.4	10

Table 2. Inter-assay variation of the Biotrin GSTYb1 EIA.

## **APPENDIX 1:**

### **EXPRESSING THE RELEASE OF GSTYB1 IN TERMS OF RATE**

In situation of unusual diuresis, e.g., poly- or oligouria, it may be more relevant to express GSTYB1 release in terms of rate (GSTYb1ng/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 GSTYb1 RELEASE RATE**

1. Determine urinary GSTYb1 levels using the Biotrin GSTYb1 EIA ( $\mu\text{g/L}$ ).
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:

$$\text{GSTYb1 ng/min} = \frac{(\text{GSYb1 } \mu\text{g/L}) \times V}{T}$$

#### **WARRANTY:**

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

## **SUMMARY OF MANUAL ASSAY PROCEDURE:**

**Note: all incubations are performed at room temperature**

1. Pipette: **100 $\mu$ L standards/positive control/sample**



Incubate: **60 min**



Wash: **4 X 300  $\mu$ L**

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



Incubate: **60 min**



Wash: **4 X 300  $\mu$ L**

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



Incubate: **15 min**

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

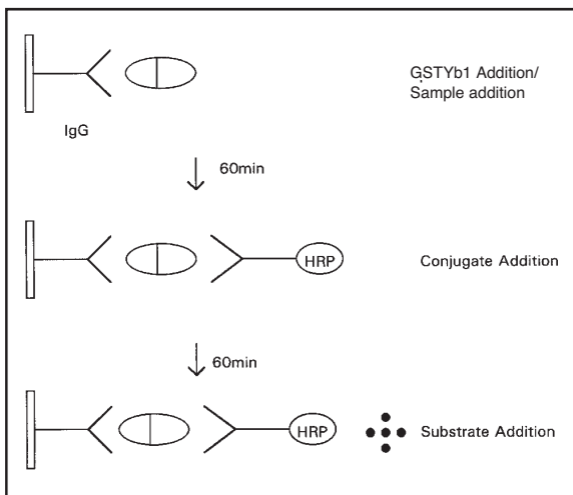


Read immediately: **450nm / 630nm**

## OTHER BIOTRIN SMARTASSAYS:

Name	For detection of	Code
HEPKIT® Alpha	$\alpha$ GST in human serum and plasma	BIO60HEPA
High Sensitivity Alpha GST EIA	$\alpha$ GST in human serum and plasma	BIO60HEPAS
Pi GST EIA	$\pi$ GST in human urine and plasma	BIO85
NEPHKIT® Alpha	$\alpha$ GST in human urine	BIO66NEPHA
RPA-1 EIA	RPA-1 (Renal Papillary Antigen 1) in rat urine	BIO89RPA1
Rat Alpha GST EIA	$\alpha$ GST in rat urine, serum and tissue culture fluid	BIO64RAT
Serum Collagen IV EIA	Collagen IV in human serum	BIO82
Urinary Collagen IV EIA	Collagen IV in human urine	BIO83
RPA-1 purified mAb	Rat renal collecting duct antigen RPA-1	BIO87CD
RPA-2 purified mAb	Rat renal loop of Henle antigen RPA-2	BIO88LH
TAP Service	Trypsinogen Activation Peptide (TAP) in human and mammalian urine	TAP
OxyDNA Test	Oxidative DNA damage in cell suspensions	BIO81DNA

### Biotrin GSTYb1 EIA: SCHEMATIC REPRESENTATION OF ASSAY PROCEDURE



## **REFERENCES:**

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3. **Coluccio, D. *et al.*** (2001). Evaluation of Biotrin Rat Alpha and Mu Glutathione S-Transferase (GST) Assays on the Biochem Immunosystems (US) Inc Labotech. Poster Presented at AACC, Meeting, Chicago, July 29 - August 3, 2001.
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6. **Davies D. *et al.*** (200). Novel Biomarkers for the Detection of Regional Kidney Damage in the Rat. Poster presented at the EMBODY Meeting, Cambridge, England, April 5-7 2000.
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