

**REF** **BIO76YB1**  
**96 Well Plate**



**ARGUTUS MEDICAL**

# **Rat GST Yb1 EIA**

**Enzyme Immunoassay**

**Instructions for Use**

**FOR RESEARCH USE ONLY**  
**Not for use in Diagnostic Procedures**

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## **INTENDED USE**

The Argutus Medical GST Yb1 EIA provides a method for the quantitative determination of rat Mu glutathione S-transferase ( $\mu$ GST, GST Yb1). For the assay of GST Yb1 in other species and other GST subclasses, contact Argutus Medical for advice.

## **BACKGROUND**

GST Yb1 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>. GST Yb1 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 GST Yb1 levels may be indicative of distal tubule damage<sup>6</sup>. The assay can be used together with Argutus Medical'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 Argutus Medical 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-GST Yb1 IgG. The resultant colour intensity is proportional to the amount of GST Yb1 present in the sample. The assay range is 1.56–100 $\mu$ g/L.

## **COMPONENTS**

- |                                                                                                                                                           |                                                                                                                                                                             |      |      |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------|-----|
| <p>1. Antibody Coated Microassay Plate:<br/>12 x 8 well strips coated with IgG directed against GST Yb1.<br/>Break apart wells.<br/>READY TO USE</p>      | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">PLA</td> </tr> </table>                                                                        | PLA  |      |     |
| PLA                                                                                                                                                       |                                                                                                                                                                             |      |      |     |
| <p>2. GST Yb1 Calibrator:<br/>Purified GST Yb1 with added stabilizers (5mg/L, 100µL).<br/>Contains Thiomersal and sodium azide.<br/>STOCK SOLUTION</p>    | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">CAL</td> </tr> </table>                                                                        | CAL  |      |     |
| CAL                                                                                                                                                       |                                                                                                                                                                             |      |      |     |
| <p>3. Sample Diluent<br/>Protein containing solution with added stabilizers (50mL).<br/>Contains Thiomersal and sodium azide.<br/>READY TO USE</p>        | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">DIL</td> <td style="padding: 5px;">SPE</td> <td style="padding: 5px;">1X</td> </tr> </table>   | DIL  | SPE  | 1X  |
| DIL                                                                                                                                                       | SPE                                                                                                                                                                         | 1X   |      |     |
| <p>4. Wash Concentrate<br/>25x Tris buffered saline/Tween-20 (TBST, 55mL).<br/>Contains Thiomersal.<br/>CONCENTRATE</p>                                   | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">BUF</td> <td style="padding: 5px;">WASH</td> <td style="padding: 5px;">25X</td> </tr> </table> | BUF  | WASH | 25X |
| BUF                                                                                                                                                       | WASH                                                                                                                                                                        | 25X  |      |     |
| <p>5. Positive Control<br/>GST Yb1 in protein containing solution with stabilisers (4.5mL)<br/>Contains Thiomersal and sodium azide.<br/>READY TO USE</p> | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">PC</td> <td style="padding: 5px;">+</td> </tr> </table>                                        | PC   | +    |     |
| PC                                                                                                                                                        | +                                                                                                                                                                           |      |      |     |
| <p>6. Conjugate<br/>Anti-rat GST Yb1 IgG conjugated to horseradish peroxidase (12mL).<br/>Contains Thiomersal.<br/>READY TO USE</p>                       | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">CONJ</td> <td style="padding: 5px;">ENZ</td> <td style="padding: 5px;">1X</td> </tr> </table>  | CONJ | ENZ  | 1X  |
| CONJ                                                                                                                                                      | ENZ                                                                                                                                                                         | 1X   |      |     |
| <p>7. Substrate<br/>Stabilised liquid TMB solution (11mL).<br/>READY TO USE</p>                                                                           | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">SUBS</td> <td style="padding: 5px;">TMB</td> </tr> </table>                                    | SUBS | TMB  |     |
| SUBS                                                                                                                                                      | TMB                                                                                                                                                                         |      |      |     |
| <p>8. Stop Solution<br/>1N Sulphuric Acid (11mL).<br/>READY TO USE</p>                                                                                    | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">SOLN</td> <td style="padding: 5px;">STP</td> </tr> </table>                                    | SOLN | STP  |     |
| SOLN                                                                                                                                                      | STP                                                                                                                                                                         |      |      |     |
| <p>9. Rat Urine Stabilising Buffer (10mL).<br/>Contains Thiomersal and sodium azide<br/>READY TO USE</p>                                                  | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">BUF</td> <td style="padding: 5px;">NEPH</td> </tr> </table>                                    | BUF  | NEPH |     |
| BUF                                                                                                                                                       | NEPH                                                                                                                                                                        |      |      |     |
| <p>10. Product Insert</p>                                                                                                                                 | <table border="1" style="margin-left: auto;"> <tr> <td style="padding: 5px;">INS</td> </tr> </table>                                                                        | INS  |      |     |
| INS                                                                                                                                                       |                                                                                                                                                                             |      |      |     |

## **PRECAUTIONS**

### **SAFETY**

- The Argutus Medical GST Yb1 EIA kit is for research use only and is not for use in diagnostic procedures.
- The Argutus Medical 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 work bench.

**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:

Stock: 20µL  
Sample Diluent: 980µL  
Total: 1000µL @ 100µg/L (A)

Using labelled test tubes, prepare further calibrators as follows:

<b>To prepare calibrator: (GST Yb1 µg/L)</b>	<b>Volumes of solutions required</b>
100 (A)	Solution A
50 (B)	Add 500µL of A to 500µL of Sample Diluent
25 (C)	Add 500µL of B to 500µL of Sample Diluent
12.5 (D)	Add 500µL of C to 500µL of Sample Diluent
6.25 (E)	Add 500µL of D to 500µL of Sample Diluent
3.13 (F)	Add 500µL of E to 500µL of Sample Diluent
1.56 (G)	Add 500µL of F to 500µL of Sample Diluent
0 (H)	Sample Diluent

**STANDARDS MUST BE USED BEFORE STATED 30MIN**

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

The Argutus Medical Rat GST Yb1 EIA can be used to measure GST Yb1 in any urine sample, but it is recommended for optimal results that timed, quantitative, urine samples are used. This will enable GST Yb1 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 Argutus Medical for advice.

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

In the absence of bacterial growth, no change in GST Yb1 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 $\mu$ L sample to 450 $\mu$ 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; 0-100µg/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 4 times with Wash Solution (**250µL-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. 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 Step 1.5.

### 3. COLOUR DEVELOPMENT

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

### 4. STOP

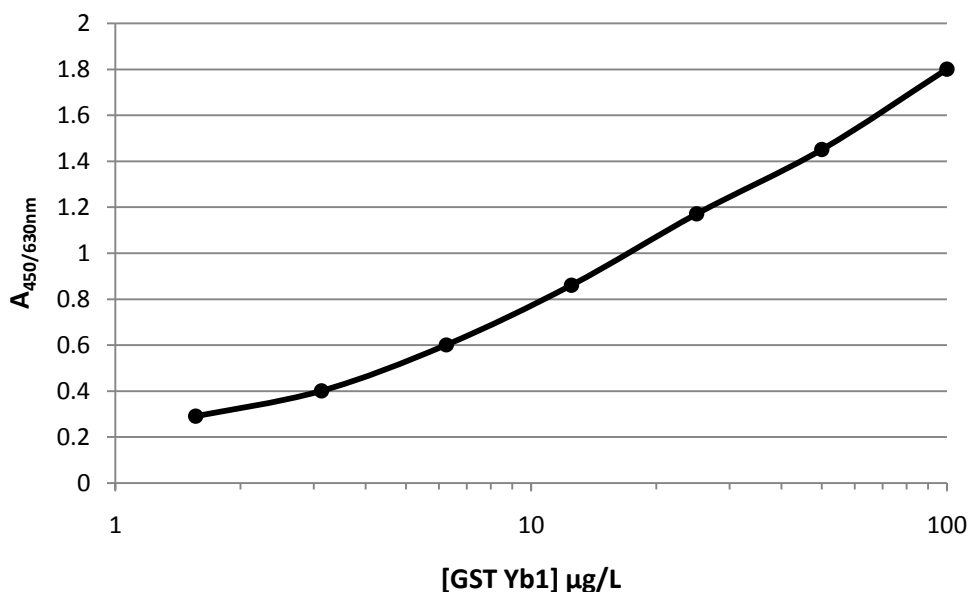
4.1. Stop the reaction by addition of **100µL** Stop Solution / well. Ensure complete mixing of Substrate and Stop Solution

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 [GST Yb1] ( $\mu\text{g/L}$ ) (Lin-log plot). (See Figure 1).
3. Read the [GST Yb1] ( $\mu\text{g/L}$ ) indicated by the mean absorbances of the samples from the Calibration curve.
4. Multiply the calculated [GST Yb1] by the appropriate dilution factor in order to obtain the actual [GST Yb1].
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 Argutus Medical 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.
8. Please see Appendix 1 for instructions on how to express urinary GST Yb1 as rate (ng/min)

## **EXAMPLE OF CALIBRATION CURVE**



**Figure 1:** Typical Calibration curve obtained using the Argutus Medical GST Yb1 EIA. Lin-log plot of A<sub>450nm/630nm</sub> versus [GST Yb1]µg/L.

## **PERFORMANCE CHARACTERISTICS**

### **NORMAL RANGE**

Normal ranges may vary between different strains of rat.

The concentration of GST Yb1 in urine from Sprague Dawley rats (n=31) was 33.9 ± 14.6µg/L ( $\bar{x} \pm S.D.$ ).

In Wistar rats (n=20), the GST Yb1 concentration was 11.4 ± 5.3µg/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 100µg/L, corresponding to 19.5 - 1250µg/L GST Yb1 in stabilised urine samples diluted 1/10. This range may be extended by increasing sample dilution.

### **LIMIT OF DETECTION**

The sample detection limit of Argutus Medical GST Yb1 EIA kit is 0.2µg/L: equivalent to 2.5µg/L in a stabilised urine sample diluted 1/10.

## SPECIFICITY

The Argutus Medical GST Yb1 EIA is highly specific for the detection of GST Yb1 ( $\mu$ GST). No significant cross-reactivity is observed with either alpha (YaYc) or pi (Yp) isoforms of rat GST.

## REPRODUCIBILITY

Sample	Mean $\mu$ g/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 Argutus Medical GST Yb1 EIA

Sample	Mean $\mu$ g/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 Argutus Medical GST Yb1 EIA

## SAMPLE RECOVERY

Recovery of added GST Yb1 was 97% over the range 30 - 100 $\mu$ g/L.

## **APPENDIX 1**

### **EXPRESSING THE RELEASE OF GST YB1 IN TERMS OF RATE**

In the situation of unusual diuresis, e.g., poly- or oligouria, it may be more relevant to express GST Yb1 release in terms of rate (GST Yb1 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 GST Yb1 levels using the Argutus Medical GST Yb1 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:

$$\text{GST Yb1 ng/min} = \frac{[\text{GST Yb1}] \mu\text{g/L} \times V}{T}$$

#### **WARRANTY**

The performance data presented here were 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: **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 solution**



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

## **REFERENCES**

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2. **Kilty, C. et al.** (1998). Glutathione S-Transferases as Biomarkers of Organ Damage: Applications of Rodent and Canine GST Enzyme Immunoassays. *Chemico-Biological Interaction*, **111-112**:123-135.
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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5. **Eger II, E.I. et al.** (1997). Nephrotoxicity of Sevoflurane versus Desflurane Anesthesia in Volunteers. *Anesthesia and Analgesia*, **84**: 160-168.
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.
7. **Okamoto, K. et al.** Evaluation of Kidney Toxicity Detection Methods in Rat Urine: GSTs by  $\alpha$  and  $\mu$ GST Enzyme Immunoassays, Metabolite Profiling by NMR and Protein Profiling by SELDI-TOF-MS. Poster presented at the 44th annual meeting of the Society of Toxicology, New Orleans, USA (2005).

## **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>BIO64RT</b>	Rat Alpha GST EIA	$\alpha$ GST in rat serum, urine and tissue culture
<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 and plasma
<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



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