

**REF** **BIO89RPA1**  
**96 well plate**



# Rat RPA-1

**Enzyme Immunoassay**

**Instructions for Use**

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

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## **INTENDED USE**

Argutus Medical Rat RPA-1 EIA is an enzyme immunoassay for the quantitative estimation of rat Renal Papillary Antigen 1 (RPA-1) in rat urine.

## **BACKGROUND**

RPA-1 is a sensitive and specific biomarker of injury to the rat renal collecting ducts. RPA-1 was discovered by screening antibodies raised against renal extracts for their ability to bind to specific renal tissues (Histomics®). The antigen is strongly expressed in the papillary collecting ducts of rat kidneys<sup>1</sup> and is released into urine upon exposure to renal toxins, e.g., bromoethanamine, propyleneamine, ipsapirone and indomethacin<sup>2</sup>. The assay can be used together with Argutus Medical's Rat Alpha GST EIA and GST Yb1 EIA, which monitor injury to the proximal and distal tubules, respectively, to provide a broad picture of injury to the renal tubules.

## **ASSAY PRINCIPLE**

The Argutus Medical Rat RPA-1 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 RPA-1 IgG. The resultant colour intensity is proportional to the amount of RPA-1 in the sample. The assay range is 3.12 -100 RPA-1 Units/L.

## COMPONENTS

Each Argutus Medical Rat RPA-1 EIA contains reagents for 96 assay wells, sufficient for 40 samples in duplicate plus a standard curve.

1. Antibody coated Microassay plate: 

PLA
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96 wells (12x8 break well strips) coated with IgG directed against rat RPA-1.  
READY TO USE
  
2. Enzyme Conjugate Concentrate: 

CONJ	ENZ	50X
------	-----	-----

  
50 X Anti-rat RPA-1 IgG conjugated to horseradish peroxidase (300µL). Contains stabiliser.  
CONCENTRATE
  
3. Enzyme Conjugate Diluent (11mL). 

CONJ	DIL	1X
------	-----	----

  
Contains ProClin 950 and Bronidox L.  
READY TO USE
  
4. RPA-1 Calibrator\*\*. 

CAL
-----

  
Partially-purified rat RPA-1 in stabiliser (1000 RPA-1 Units/L; 500µL). Contains ProClin 950 and Bronidox L. **Store at -20°C until required.**  
STOCK SOLUTION
  
5. Positive Control\*\*. 

PC	25X
----	-----

  
25X Partially-purified rat RPA-1 in stabiliser (100µL). Contains ProClin 950 and Bronidox L.  
**Store at -20°C until required.**  
CONCENTRATE
  
6. Rat Urine Stabilising Buffer (10mL). 

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

  
Contains Thiomersal and Sodium Azide.  
READY TO USE
  
7. Sample Diluent (50mL) 

DIL	SPE	1X
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Contains Stabiliser and ProClin 950.  
READY TO USE
  
8. Wash Concentrate: 

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

  
25X Tris-buffered saline/Tween-20 (TBST; 55mL). Contains Trizma base and ProClin 950.  
CONCENTRATE
  
9. Substrate: 

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

  
Stabilised liquid TMB solution (11mL)  
READY TO USE
  
10. Stop Solution: 

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

  
1N Sulphuric Acid (11mL)  
READY TO USE
  
11. Instructions for use 

INS
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\*\* Potentially Biohazardous Material

## **PRECAUTIONS**

### **SAFETY**

- The Argutus Medical Rat RPA-1 EIA kit is for research use only and is not for use in diagnostic procedures.
- The Argutus Medical Rat RPA-1 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.
- The Stabilising Buffer contains 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 a 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.
- 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 **except for the RPA-1 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.
2. RPA-1 calibrators and positive control must be used within one hour of preparation.
3. Diluted conjugate must be used within one hour 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.
5. Microassay wells should be stored in sealed foil pouches with desiccants at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.

## **ADDITIONAL MATERIALS REQUIRED**

**Note:** for manual operation of assay.

1. Micropipettes (5 $\mu$ L to 50 $\mu$ L, 50 $\mu$ L to 200 $\mu$ L and 200 $\mu$ L to 1000 $\mu$ L) and a multichannel pipette (50 $\mu$ L to 200 $\mu$ L)
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. Room temperature incubator
12. ELISA plate reader capable of reading at 450nm (using 630nm as reference if possible)

## **PREPARATION OF REAGENTS**

### **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 45mL of Wash Solution.

### **CONJUGATE**

Prepare a 1/50 dilution of Conjugate Concentrate by adding, for example, 20 $\mu$ L Conjugate Concentrate to 980 $\mu$ L Enzyme Conjugate Diluent. Each strip of 8 microassay wells requires a minimum of 800 $\mu$ L of diluted Conjugate. Diluted Conjugate is stable for at least 60 min following preparation.

### **CALIBRATORS**

Dilute the RPA-1 Calibrator Stock solution (1000 RPA-1 Units/L) thus:

Stock:	50 $\mu$ L
Sample Diluent:	<u>450<math>\mu</math>L</u>
Total:	500 $\mu$ L @ 100 RPA-1 Units/L ( <b>Solution A</b> )

Using labelled test tubes, prepare calibrators as follows:

To prepare calibrator: (RPA-1 Units/L)	Volumes of solutions required
A (100)	Solution A
B (50)	Add 250µL of A to 250µL of Sample Diluent
C (25)	Add 250µL of B to 250µL of Sample Diluent
D (12.5)	Add 250µL of C to 250µL of Sample Diluent
E (6.25)	Add 250µL of D to 250µL of Sample Diluent
F (3.13)	Add 250µL of E to 250µL of Sample Diluent
G (0)	Add 250µL of Sample Diluent

**Standards must be used within one hour of preparation.**

### **POSITIVE CONTROL**

Dilute Positive Control 1/25 in Sample Diluent, i.e., add 20µL Positive Control to 480µL Sample Diluent. Diluted Positive Control must be used within one hour of preparation

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

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

As soon as possible after sample collection, add 200µL of Rat Urine Stabilising Buffer to 800µL 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 αGST and GST Yb1 using the respective Argutus Medical assays.

Samples can be stored at 2-8°C for 48 hours or at -20°C for at least 1 year. Repeated freeze thawing should be avoided.

### **SAMPLE PREPARATION**

Dilute stabilised urine samples 1/25 in Sample Diluent, e.g., add 20µL urine to 480µL Sample Diluent. Diluted samples should be used within one hour of preparation and should not be stored.

## **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 each wash step, remove any remaining drops by tapping the Microassay plate hard against paper towels until no further liquid is deposited onto the towels. Avoid contact between the towels and the inside of the wells.
- Add next reagent promptly.

### **1. CALIBRATOR / SAMPLE INCUBATION**

1.1. Prepare the Wash Solution, Calibrators and Positive Control as described in “Preparation of Reagents”.

1.2. Prepare Samples as outlined in “Sample Preparation”.

1.3. Place the required number of microassay wells in the assay plate (14 for the calibrators, 2 for the positive control and 2 per sample). Arrange in columns of 8 and fill up spaces in the column with blank microassay wells. Add Calibrators (**G-A; 0-100 RPA-1 Units/L**), Positive Control (**100µL/well**) 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 can be used at speed 2-3.

1.5. Prepare the Conjugate 5 min before the end of the 60 min incubation period, as described in “Preparation of Reagents”.

1.6. Remove cover and wash each strip 6 times with Wash Solution (**250-320µL/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 can be used at speed 2-3.

2.3. Wash each strip as in 1.6 above.

### 3. COLOUR DEVELOPMENT

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

### 4. STOP

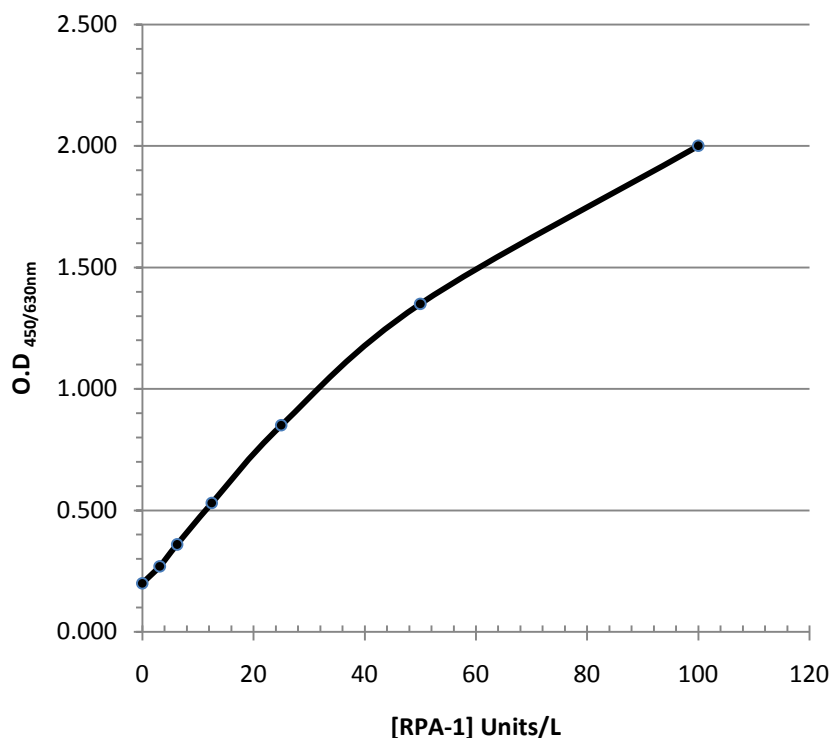
4.1. Stop the reaction by addition of **100µL** Stop Solution/well. Ensure complete mixing of Substrate and Stop Solution.

4.2. Read immediately at 450nm with 630nm as reference if available.

## CALCULATION OF RESULTS

1. Calculate the mean absorbance for each calibrator, positive control and samples.
2. Plot a calibration curve of  $A_{450/630nm}$  versus [RPA-1] (Units/L) on a linear-linear scale (see Figure 1).
3. Read the RPA-1 concentration (Units/L), indicated by the mean of the absorbance of the sample, from the calibration curve.
4. Multiply the RPA-1 concentration obtained by the appropriate dilution factor in order to obtain the actual RPA-1 concentration. Results for urine samples should be multiplied by an additional 1.25 to compensate for the dilution of sample with Rat Urine Stabilising Buffer.
5. 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.
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.
7. Please see Appendix 1 for instructions on how to express urinary RPA-1 release as rate (Units/min).

## **EXAMPLE OF A CALIBRATION CURVE**



**Figure 1:** Typical Calibration curve obtained using the Argutus Medical Rat RPA-1 EIA. OD<sub>450/630nm</sub> versus [RPA-1] Units/L, were plotted using a Linear Cubic Spline Fit with Tails.

## **PERFORMANCE CHARACTERISTICS**

### **REFERENCE RANGES**

Typical RPA-1 concentrations in the urine of normal rats have been observed in the range of 100–1300 U/L. Normal ranges may vary between different rat colonies and strains. Therefore, it is important for each laboratory to develop a reference range appropriate to the study in question. Contact Argutus Medical for advice.

### **LIMIT OF DETECTION**

The sample detection limit of Argutus Medical rat RPA-1 EIA kit is 3.12 U/L: equivalent to 97.5 U/L in a stabilised urine sample diluted 1/25.

### **MEASURING RANGE**

The calibration curve range covers 3.12–100 RPA-1 Units/L, corresponding to 97.5–3125 RPA-1 Units/L in stabilised urine samples diluted 1/25. This range may be extended by increasing sample dilution.

## SPECIFICITY

The RPA-1 Antibody binds to an antigen found in the collecting ducts of rat kidneys in frozen and formalin-fixed sections. Reaction with papillary collecting ducts is stronger than with medullar and cortical collecting ducts. No reactivity was noted with the rat loop of Henle.

## INTERFERENCE

No significant interference has been observed in this assay with up to 5 g/dL hemoglobin, 5mg/dL conjugated bilirubin, 10 g/dL NaCl, 1 g/dL Rat albumin and 50 mg/dL Rat IgG. Please contact Argutus Medical for further information.

## REPRODUCIBILITY

**Intra-assay variation** within the Argutus Medical rat RPA-1 Kit standard curve range

Sample	Mean RPA-1 U/L	SD	%CV	N
Low	17	0.4	2	24
Medium	61	3	5	24
High	90	5	6	24
PC	55	3	5	32

**Inter-assay variation** within the Argutus Medical rat RPA-1 Kit standard curve range

Sample	Mean RPA-1 U/L	SD	%CV	N
Low	18	2	11	20
Medium	60	7	11	20
High	101	8	8	20
PC	52	3	6	29

**Inter-batch variation** within the Argutus Medical rat RPA-1 Kit standard curve range calculated over three batches of kits.

Sample	Mean RPA-1 U/L	SD	%CV	N
Low	22	3	14	22
Medium	39	3	8	22
High	100	9	9	22

## RECOVERY

The recovery of the Argutus Medical rat RPA-1 EIA kit is 100% ± 20%.

## **APPENDIX 1**

### **EXPRESSING THE RELEASE OF RPA-1 IN TERMS OF RATE**

In the situation of unusual diuresis, e.g., poly- or oligouria, it may be more relevant to express RPA-1 release in terms of rate (RPA-1 Units/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 RPA-1 RELEASE RATE**

1. Determine urinary RPA-1 levels using the Argutus Medical Rat RPA-1 EIA (Units/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:

$$\text{RPA-1 mUnits/min} = \frac{[\text{RPA-1}] \text{ Units/L} \times V}{T}$$

## **WARRANTY**

The performance data presented here was obtained using the procedure described. Any change or modification in the procedure not recommended by Argutus Medical may affect the results, in which case Argutus Medical disclaims all warranties, expressed, implied or statutory, including the 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

## REFERENCES

1. **Falkenberg F. W. et al.** (1996). Urinary antigens as markers of papillary toxicity. I. Identification and characterization of rat kidney papillary antigens with monoclonal antibodies. Arch Toxicol. **71**, 80-92. **Note:** the anti-RPA-1 monoclonal was called PAP X5C10 and RPA-1, PAP1.
2. **Hildebrand, H. et al.** (1999). Urinary antigens as markers of papillary toxicity. II. Application of monoclonal antibodies for the determination of papillary antigens in rat urine. Arch. Toxicol. **73**, 233-245.

## **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>BIO76YB1</b>	Rat GST Yb1 EIA	GST Yb1 ( $\mu$ GST) 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>	NEPKIT® 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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