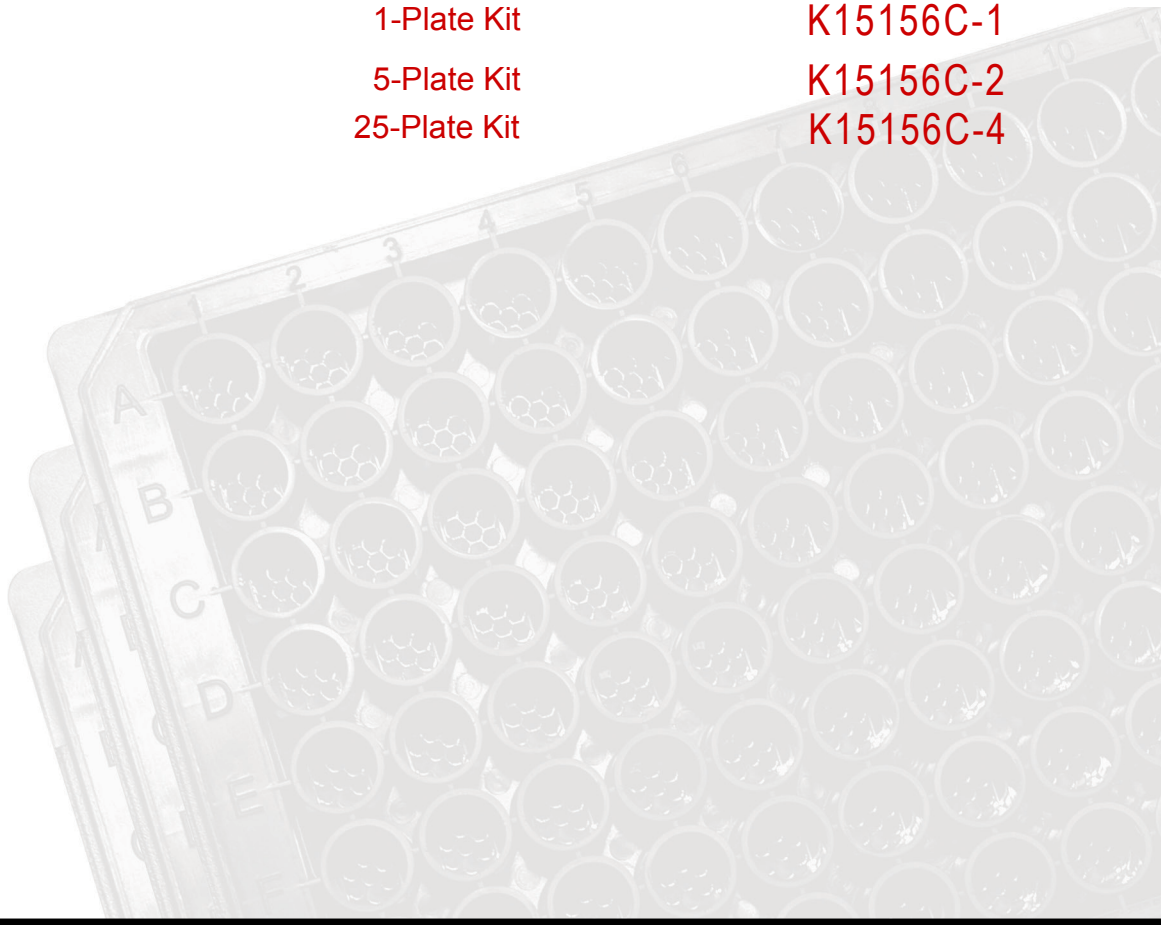
# MESO SCALE DISCOVERY

## MULTI-SPOT Assay System

### Argutus AKI Test™ (rat) Assay Kit

| | |
|---|---|
| 1-Plate Kit | K15156C-1 |
| 5-Plate Kit | K15156C-2 |
| 25-Plate Kit | K15156C-4 |



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# MSD Toxicology Assays

## Argutus AKI Test™ (rat) Assay Kit

### αGST, GSTYb1, RPA-1

*This package insert must be read in its entirety before using this product.*

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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# Ordering Information

ordering information

## MSD Customer Service


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## MSD Scientific Support


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# MSD Advantage

MSD advantage

MESO SCALE DISCOVERY'S MULTI-ARRAY® Technology is a multiplex immunoassay system that enables the measurement of biomarkers utilizing the next generation of electrochemiluminescent detection. In an MSD® assay, specific Capture Antibodies for the analytes are coated in arrays in each well of a 96-well carbon electrode plate surface. The detection system uses patented SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT® plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable and are non-radioactive. Additionally, only labels near the electrode surface are detected, enabling non-washed assays.

One of the advantages of MSD assays is the minimal sample volume required as compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, ten different biomarkers can be analyzed simultaneously using as little as 10-25 µL of sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Further, the simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell signaling pathways, and other applications, as well as a variety of plates and reagents for assay development.

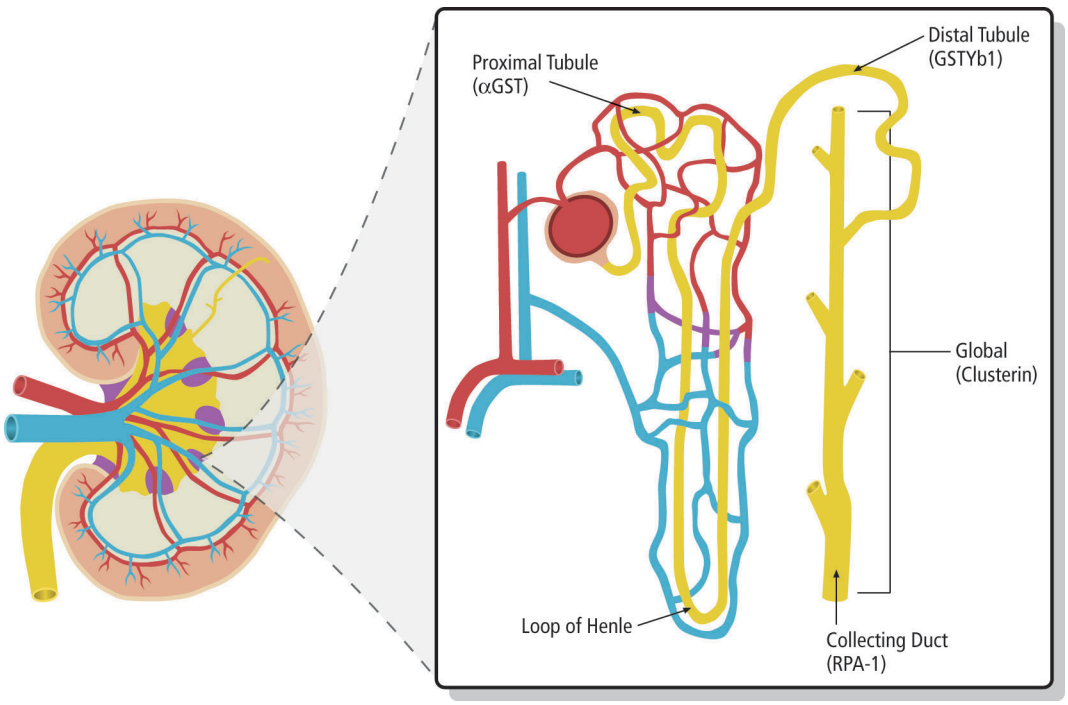
# Introduction

introduction

This product insert describes a multiplex panel of rat kidney biomarkers from Argutus Medical Limited (formerly Biotrin International). Argutus AKI Test (rat) includes αGST, GSTYb1, and RPA-1, the combination of which allows researchers to stratify acute kidney injury between specific cell types and pinpoint the site of injury.

**Glutathione S-transferases (GSTs)** are proteins found in high concentrations (2% of soluble protein) in the luminar cells of the proximal and distal tubules. Different isoforms are found in different parts of the nephron, therefore, by measuring urinary alpha GST (αGST) one can study the proximal tubule and, by measuring GSTYb1 in rats, one can study the distal tubule. Renal tubular injury can thus be precisely localized. These proteins are not released in the healthy rat and as such act as a very sensitive indicator of site specific injury.

**Renal Papillary Antigen 1 (RPA-1)** is the first urinary biomarker for injury to the luminal epithelial cell of the collecting duct. Injury to the collecting ducts in the renal papilla can lead to the Renal Papillary Necrosis (RPN), a serious condition for which urinary biomarkers are currently lacking.



# Principle of the Assay

principle of the assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. These assays have been qualified according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al[1]. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Argutus AKI Test (rat) Assay is a multiplexed sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with three capture antibodies on spatially distinct spots—antibodies for αGST, GSTYb1, and RPA-1. The user adds the sample and a solution containing the labeled detection antibodies—Anti-rat αGST, Anti-rat GSTYb1, and Anti-rat RPA-1 labeled with an electrochemiluminescent compound, MSD SULFO-TAG label—over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibodies by bound analytes completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument for analysis. Inside the MSD instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of αGST, GSTYb1, and RPA-1 present in the sample.

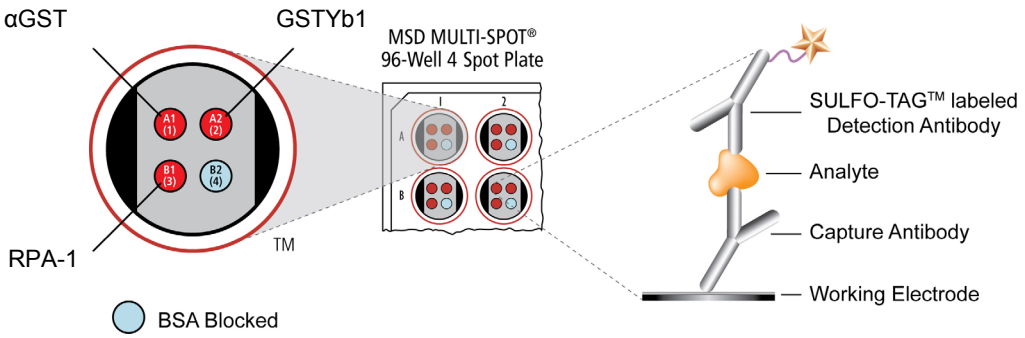



***Figure 1.*** *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

# IV Reagents Supplied

reagents supplied

| Product Description | Storage | Quantity per Kit K15156C-1 | K15156C-2 | K15156C-4 |
|---|---|---|---|---|
| MULTI-SPOT 96-well Argutus AKI Test (rat) Plate<br>N45156A | 2-8°C | 1 plate | 5 plates | 25 plates |
| SULFO-TAG Anti-rat αGST Antibody (50X)[1] | 2-8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| SULFO-TAG Anti-rat GSTYb1 Antibody (50X)[1] | 2-8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| SULFO-TAG Anti-rat RPA-1 Antibody (50X)[1] | 2-8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| Argutus AKI Test (rat) Calibrator Blend | ≤ -70°C | 1 vial<br>(200 µL) | 5 vials<br>(200 µL ea) | 25 vials<br>(200 µL ea) |
| Blocker B | RT | 1 bottle<br>(1 g) | 1 bottle<br>(1 g) | 5 bottles<br>(1 g ea) |
| Diluent 31<br>R50IA-4 (15 mL), R50IA-9 (60 mL) | ≤ -10°C | 1 bottle<br>(15 mL) | 1 bottle<br>(60 mL) | 5 bottles<br>(60 mL ea) |
| Read Buffer T (with surfactant), 4X<br>R92TC-3 (50 mL) | RT | 1 bottle<br>(50 mL) | 1 bottle<br>(50 mL) | 5 bottles<br>(50 mL ea) |

# V Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate-buffered saline (PBS) for making Blocker B solution
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

# VI Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

# VII Reagent Preparation

reagent preparation

Bring all reagents to room temperature.

> **Important:** Upon first thaw, separate Diluent 31 into aliquots appropriate to the size of your assay needs. The diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

## Prepare Blocking Solution

Prepare a solution of 0.5% (w/v) Blocker B in 1X PBS to be used as a blocking solution. For one plate, mix 20 mL of PBS with 0.1 g of Blocker B. Mix at room temperature until all the Blocker B has dissolved. 0.15 mL per well of blocking solution will be required.

## Prepare Calibrator and Control Solutions

Calibrators for the Argutus AKI Test (rat) are supplied at the concentration of the highest calibrator. No dilution is required for the highest calibrator. For each assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a Zero Calibrator. The Calibrators are supplied as a blend. The stock Calibrator blend should be thawed and kept on ice, but should be added into diluent at room temperature to make the standard curve solutions. For the actual concentrations of each Calibrator, refer to the certificate of analysis (C of A) supplied with the kit.

To prepare this 8-point standard curve:

1) The highest Calibrator is the undiluted calibrator blend.
2) Prepare the next Calibrator by transferring 80 µL of the highest Calibrator to 160 µL of Diluent 31. Repeat 3-fold serial dilutions 6 additional times to generate 7 Calibrators.
3) The recommended 8$^{th}$ Standard is Diluent 31 alone (i.e. Zero Calibrator).

Calibrators should be prepared no more than 20 minutes before use.

## Dilution of Samples

Some rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. If this is the case, Diluent 31 should be used to dilute samples. For rat urine samples, a 5-fold dilution is recommended. Samples with high analyte abundance may need further dilution. For serum, plasma, or tissue homogenates a 5–5000-fold dilution may be required.

## Prepare Detection Antibody Solution

The Detection Antibodies are provided as a 50X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X.

In a 15 mL tube combine (per plate):

- ❑ 60 µL of 50X SULFO-TAG Anti-rat αGST Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat GSTYb1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-rat RPA-1 Antibody
- ❑ 2820 µL of Diluent 31

**Important:** Prepare the Detection Antibody Solution no longer than 15 minutes prior to addition to the plate.

## Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

## Prepare MSD Plate

This plate has been pre-coated with antibodies for the analytes shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

# VIII Assay Protocol

assay protocol

***Notes***

1. **Addition of Blocking Solution:** Dispense 150 µL of 0.5% Blocker B Solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

*Shaking a 96-well MSD MULTI-SPOT plate typically accelerates capture at the working electrode.*

2. **Wash and Addition of the Sample or Calibrator:** Wash the plate 3 times with PBS-T. First, dispense 25 µL of Diluent 31 into each well of the MSD plate. Then, dispense 25 µL of sample or calibrator into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3 times with PBS-T. Dispense 25 µL into each well of the MSD plate within 15 minutes of 1X Detection Antibody Solution preparation. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3 times with PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. ***After adding Read Buffer, keep the plate at room temperature without shaking for 5 minutes before analyzing the plate on the MSD instrument.***

*Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.*

# IX Analysis of Results

analysis of results

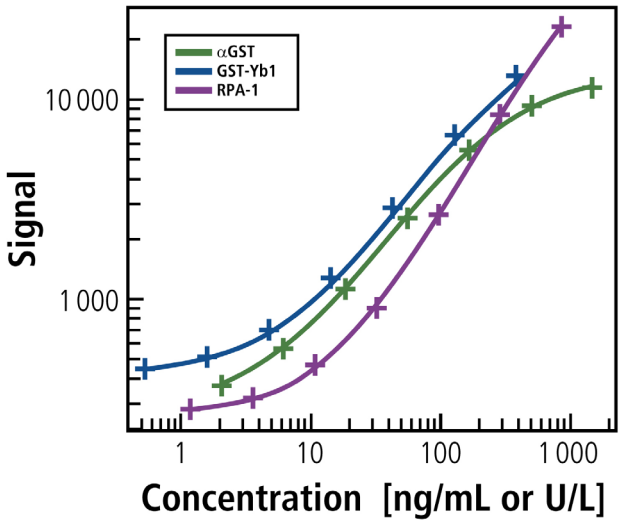
The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

# Typical Standard Curve

typical standard curve

**Below are representative data for this assay. The actual kit specific standard curve can be found in the certificate of analysis enclosed with the kit.**

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples. Calibration curves are lot specific since they are made using papilla- medulla extracts.




| αGST | | |
|---|---|---|
| Conc. (ng/mL) | Average Counts | %CV |
| 0 | 270 | 6.2 |
| 2.1 | 371 | 4.1 |
| 6.2 | 568 | 4.2 |
| 18.5 | 1122 | 3.7 |
| 55.6 | 2573 | 2.8 |
| 167 | 5583 | 2.6 |
| 500 | 9299 | 3.1 |
| 1500 | 11394 | 2.7 |

| GSTYb1 | | |
|---|---|---|
| Conc. (ng/mL) | Average Counts | %CV |
| 0 | 419 | 3.7 |
| 0.6 | 451 | 3.8 |
| 1.8 | 519 | 3.4 |
| 5.3 | 706 | 3.2 |
| 15.9 | 1271 | 3.3 |
| 47.8 | 2906 | 3.1 |
| 143 | 6630 | 1.6 |
| 430 | 13167 | 2.9 |

| RPA-1 | | |
|---|---|---|
| Conc. (U/L) | Average Counts | %CV |
| 0 | 263 | 4.5 |
| 1.2 | 280 | 4.0 |
| 3.6 | 320 | 4.2 |
| 10.7 | 467 | 3.0 |
| 32.2 | 908 | 4.2 |
| 96.7 | 2676 | 4.7 |
| 290 | 8346 | 2.9 |
| 870 | 22886 | 3.7 |

# XI Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%. For RPA-1, the percent recovery is between 75% and 125%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%. For RPA-1, the percent recovery is between 75% and 125%.

| | αGST (ng/mL) | GSTYb1 (ng/mL) | RPA-1 (U/L) |
|---|---|---|---|
| **LLOD** | 0.87 | 0.71 | 2.0 |
| **LLOQ** | 6.6 | 12.4 | 30 |
| **ULOQ** | 196 | 333 | 800 |

# XII Precision

precision

A set of four control samples was measured on each plate. Controls were made by spiking papilla medulla extract into rat urine.

| | Control | Plates | Avg Conc. | Average Intra-plate % CV | Inter-plate % CV |
|---|---|---|---|---|---|
| **αGST (ng/mL)** | 3 | 8 | 79.1 | 2.9 | 6.5 |
| | 4 | 8 | 61.7 | 3.3 | 6.6 |
| **GSTYb1 (ng/mL)** | 2 | 8 | 86.5 | 4.1 | 5.1 |
| | 3 | 8 | 28.4 | 4.3 | 5.7 |
| **RPA-1 (U/L)** | 1 | 8 | 471 | 4.7 | 5.6 |
| | 2 | 8 | 89.6 | 5.5 | 6.3 |

# XIII Spike Recovery

spike recovery

Papilla medulla extract was spiked into 5X diluted rat urine and tested on the Argutus AKI Test. The concentrations of the spikes were distributed throughout the linear range of the assay. All of the spiked samples above the LLOQ had acceptable recoveries (between 80% and 120%).

% Recovery = measured / expected x 100

| | Spike Level | Conc. | Conc. %CV | % Recovery |
|---|---|---|---|---|
| αGST (ng/mL) | 0 | 7.8 | 1.5 | |
| | 13.9 | 22.8 | 8.6 | 105 |
| | 41.7 | 46.7 | 1.0 | 94 |
| | 125 | 132 | 7.0 | 100 |
| GSTYb1 (ng/mL) | 0 | 2.9 | 111 | |
| | 27.8 | 28.8 | 8.6 | 94 |
| | 83.3 | 90.2 | 22.2 | 105 |
| | 250 | 239 | 4.4 | 95 |
| RPA-1 (U/L) | 0 | 114 | 8.5 | |
| | 27.8 | 138 | 7.2 | 98 |
| | 83.3 | 202 | 6.1 | 102 |
| | 250 | 345 | 6.2 | 95 |

# XIV Linearity

linearity

Two normal, Sprague Dawley rat urine samples were diluted to measure linearity. Sample 1 was below the quantitative range for GSTYb1, and sample 2 was below the quantitative range for αGST. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration measured from the previous dilution (expected).
% Recovery = (measured x dilution factor) / expected x 100

| | | α GST | | | GSTYb1 | | |
|---|---|---|---|---|---|---|---|
| | Fold Dilution | Conc. (ng/mL) | Conc. % CV | % Recovery | Conc. (ng/mL) | Conc. % CV | % Recovery |
| Sample 1 | 2.5 | 88.9 | 2.0 | | - | - | |
| | 5 | 90.3 | 11.2 | 102 | - | - | - |
| | 10 | 90.8 | 4.2 | 101 | - | - | - |
| Sample 2 | 2.5 | - | - | | 617 | 4.7 | |
| | 5 | - | - | - | 651 | 21.4 | 105 |
| | 10 | - | - | - | 548 | 3.2 | 84 |
| | 20 | - | - | - | 472 | 3.6 | 86 |

| | | RPA-1 | | |
|---|---|---|---|---|
| | Fold Dilution | Conc. (U/L) | Conc. % CV | % Recovery |
| Sample 1 | 5 | 1870 | 0.3 | - |
| | 10 | 1414 | 1.9 | 76 |
| | 20 | 1274 | 2.5 | 90 |
| | 40 | 1209 | 4.4 | 95 |
| Sample 2 | 5 | 1994 | 3.4 | - |
| | 10 | 1697 | 4.0 | 85 |
| | 20 | 1498 | 4.0 | 88 |
| | 40 | 1452 | 4.5 | 97 |

# XV Specificity

specificity

In order to assess specificity of the detection antibodies, the Argutus AKI Test (rat) Kit was run with blended Calibrator diluted to STD-02, and single detection antibodies. The table below shows the % cross-reactivity for each individual detection antibody.

| | Blended Calibrator and Single Detection Antibody % Cross-Reactivity | | |
|---|---|---|---|
| Spot | αGST | GSTYb1 | RPA-1 |
| αGST | **100** | 7.22 | 0.20 |
| GSTYb1 | 10.36 | **100** | 0.72 |
| RPA-1 | 0.39 | < 0.1 | **100** |

# XVI Samples

samples

Rat urine samples from animals treated with known injury inducing drugs were run at a 5-fold dilution. We confirmed high levels of RPA-1 in samples from animals treated with NPAA. Tenidap has been shown to induce elevated levels of Clusterin, another emerging biomarker of injury to the collecting duct. The MSD multiplex panel found that RPA-1 is also elevated upon treatment with Tenidap, supporting the expectation that RPA-1 is related to injury of the collecting duct. Our panel confirmed elevated levels of αGST in Cisplatin treated animals. The measurements made with our multiplex were in agreement with the concentrations determined from the Argutus EIA kits. Measurements in italics were below the assay LLOQ at a 5-fold dilution. Measurements in bold were made at a 20-fold dilution of the urine samples.

| MSD Assay Kits | | | αGST | | GSTYb1 | | RPA-1 | |
|---|---|---|---|---|---|---|---|---|
| Nephro-toxicant | Associated Biomarker | Sample ID | Conc. (ng/mL) | Conc. %CV | Conc. (ng/mL) | Conc. %CV | Conc. (U/L) | Conc. %CV |
| Control | None | B671 | 11.7 | 2.9 | *13.4* | *35.6* | 714 | 1.4 |
| | | B672 | 26.8 | 16.0 | *16.7* | *20.5* | 1382 | 2.6 |
| NPAA | RPA-1 | B673 | *7.1* | *3.1* | *29.0* | *13.1* | **8700** | **9.8** |
| | | B674 | *7.3* | *13.3* | *14.9* | *34.7* | **5482** | **5.5** |
| Tenidap | Clusterin | B675 | 20.3 | 7.6 | *23.4* | *7.2* | 4363 | 9.1 |
| | | B676 | *7.8* | *7.8* | *19.5* | *37.6* | 1657 | 5.2 |
| Cisplatin | αGST | B680 | 69.2 | 3.9 | *38.0* | *36.6* | 757 | 6.3 |
| | | B681 | 176 | 2.6 | *43.3* | *30.2* | 704 | 4.4 |

| ARGUTUS™ EIA Kits | | | αGST | GSTYb1 | RPA-1 |
|---|---|---|---|---|---|
| Nephro-toxicant | Associated Biomarker | Sample ID | Conc. (ng/mL) | Conc. (ng/mL) | Conc. (U/L) |
| Control | None | B671 | 14.0 | 4.0 | 831 |
| | | B672 | 30.0 | 3.0 | 1429 |
| NPAA | RPA-1 | B673 | 35.0 | 7.0 | 5588 |
| | | B674 | 12.0 | 3.0 | 4499 |
| Tenidap | Clusterin | B675 | 14.0 | 4.0 | 2939 |
| | | B676 | 12.0 | 6.0 | 1950 |
| Cisplatin | αGST | B680 | 138 | 12.0 | 668 |
| | | B681 | 246 | 18.0 | 663 |

# XVI Calibrators

calibrators

Papilla-medulla extract from rat is combined with recombinant GSTYb1 to make the Argutus AKI Test (rat) Calibrator Blend. The papilla-medulla extract contains all three analytes. Each lot of the Calibrator Blend is assigned a concentration for each analyte based on a multi-day test. Because of variability between batches of the Papilla-medulla extract, the concentration values of the calibrator are lot-specific.

# XVIII References

references


1. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brien PJ, Sailstad J, Khan M, Ray C, Wagner JA. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28.
2. Campbell J.A.H., *et al.* Immunohistologic localization of alpha, mu and pi class glutathione S-transferase in human tissues. Cancer. 1991;67:1608-1613.
3. Hassett, B. and Doyle, S. 1995; Biotrin International internal research.
4. Falkenberg FW, *et al*. Urinary antigens as markers of papillary toxicity identification and characterization of rat kidney papillary antigens with monoclonal antibodies. Arch Toxicol. 1996;71:80-92. *Note:* the anti-RPA-1 monoclonal was called PAP X5C10 and RPA-1, PAP1.

*Summary Protocol*

# MSD 96-well MULTI-SPOT Argutus AKI Test™ (rat) Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing
the Argutus AKI Test (rat) Assay.

**Step 1 : Sample and Reagent Preparation**

Bring appropriate diluents and plates to room temperature.

Rat urine samples should be diluted 5-fold in Diluent 31. Rat serum, plasma and tissue homogenates may require 5–5000X fold dilution in Diluent 31.

Prepare Blocking Solution by diluting Blocker B to 0.5% (w/v) in PBS.

Prepare an 8-point standard curve using supplied calibrators:

- The Calibrator Blend should be diluted in Diluent 31.
- Calibrator is supplied as 1X solution, ready to be used as the highest Calibrator of the standard curve. Reserve 160 µL of the Calibrator Blend as Calibrator 1 and dilute the remaining 80 µL by six, 3-fold serial dilution steps and a no Calibrator blank.

Prepare a Detection Antibodiy Solution by diluting the supplied Detection Antibodies stock to 1X concentration of each antibody in Diluent 31 (per plate). Each Detection Antibody is supplied as 50X stock solution. The Detection Antibody Solution should be prepared no longer than 15 minutes prior to addition to the plate.

Prepare 20 mL of 1X Read Buffer T by diluting MSD Read Buffer T with deionized water.

**Step 2 : Add Blocking Solution**

Dispense 150 µL/well Blocking Solution (0.5% Blocker B).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

**Step 3 : Wash and Add Sample or Calibrator**

Wash plate 3 times with PBS-T.

Dispense 25 µL/well Diluent 31.

Dispense 25 µL/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

**Step 4 : Wash and Add Detection Antibody Solution**

Wash plate 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution within 15 minutes of solution preparation.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

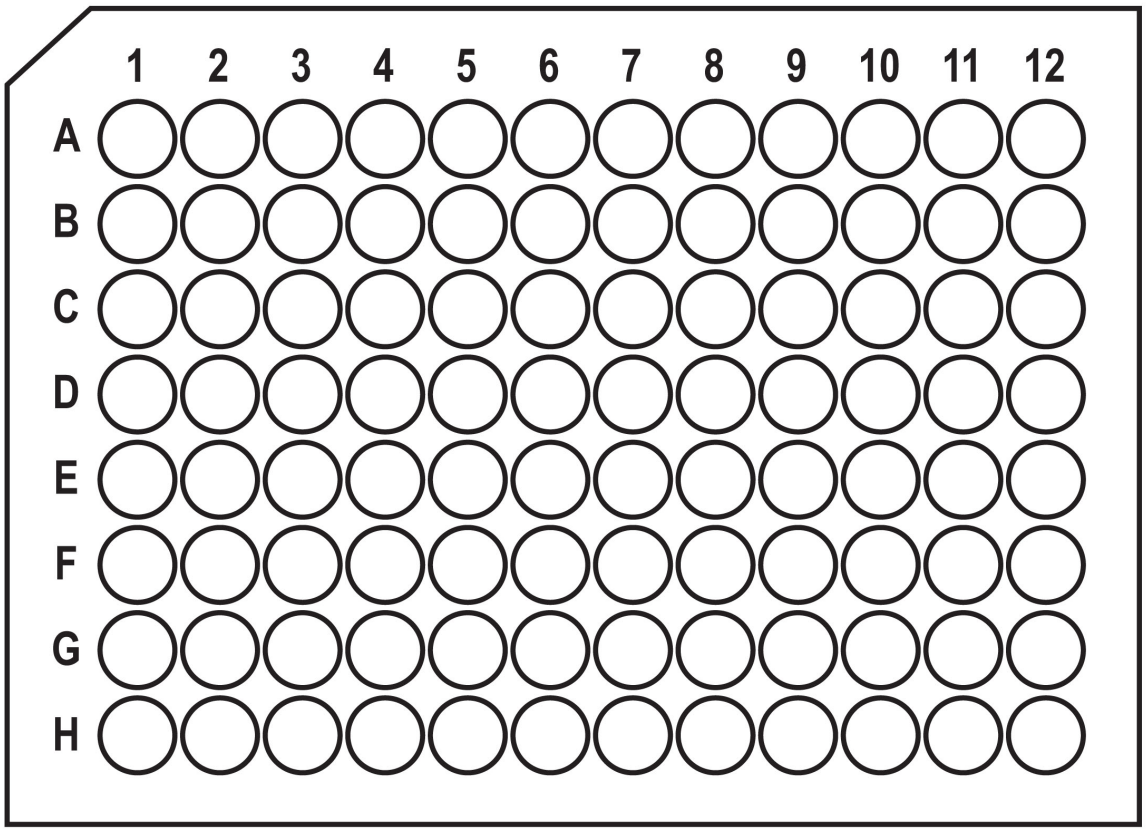
**Step 5 : Wash and Read Plate**

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 1X Read Buffer T.

Wait 5 minutes.

Analyze plate on the MSD instrument.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
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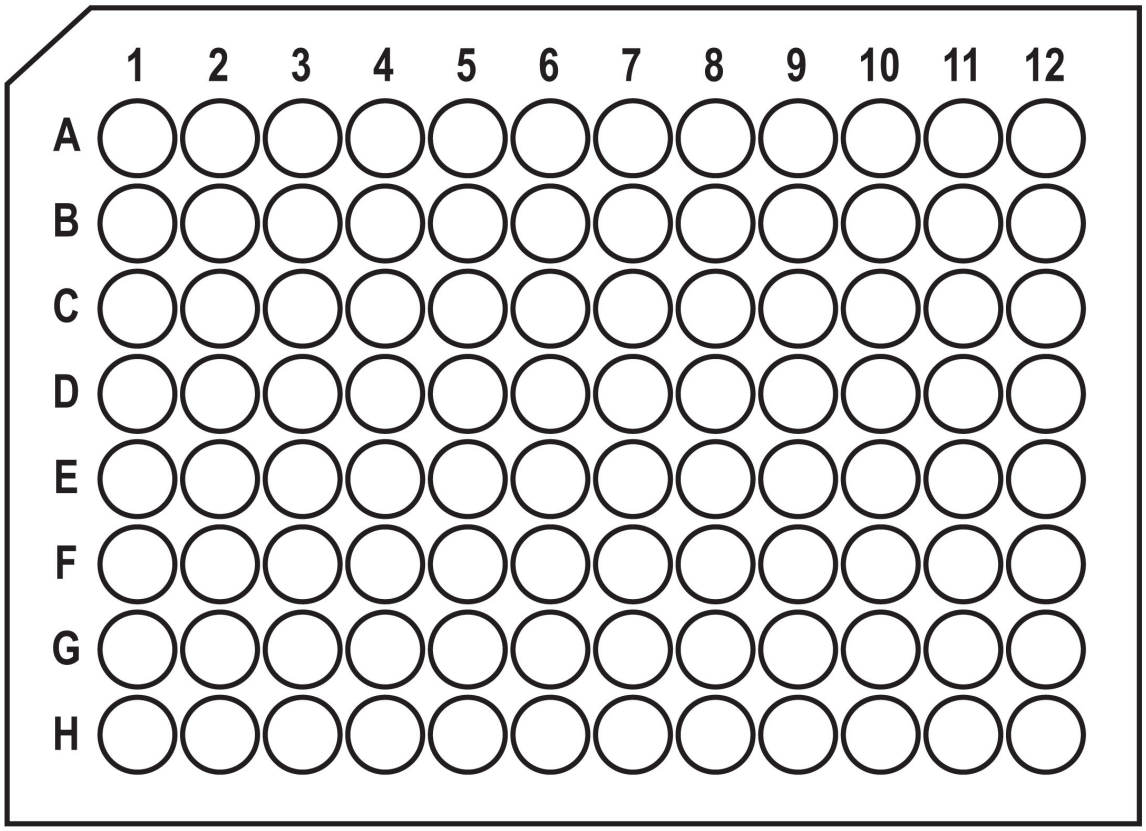
E
F
G
H


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H