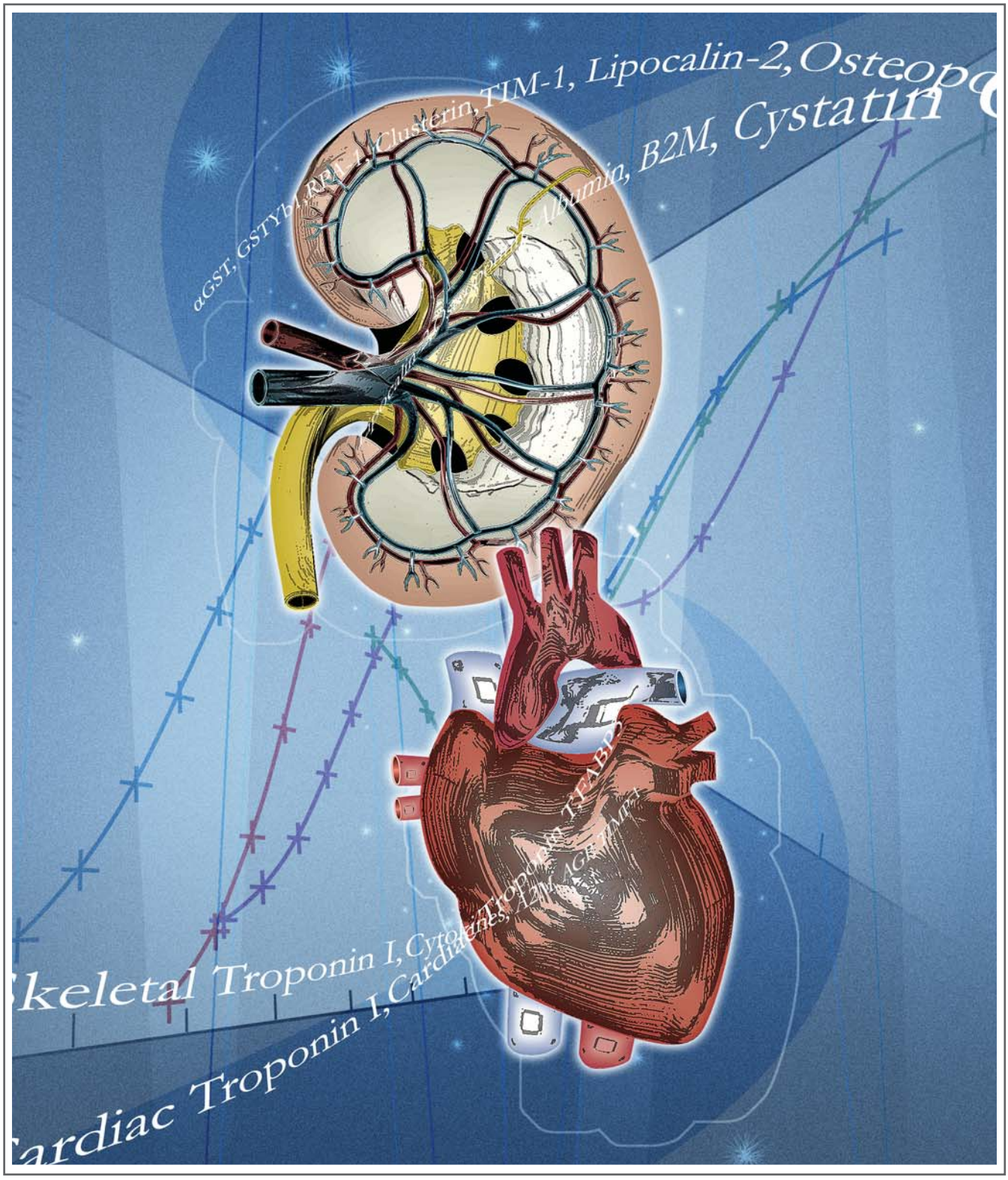
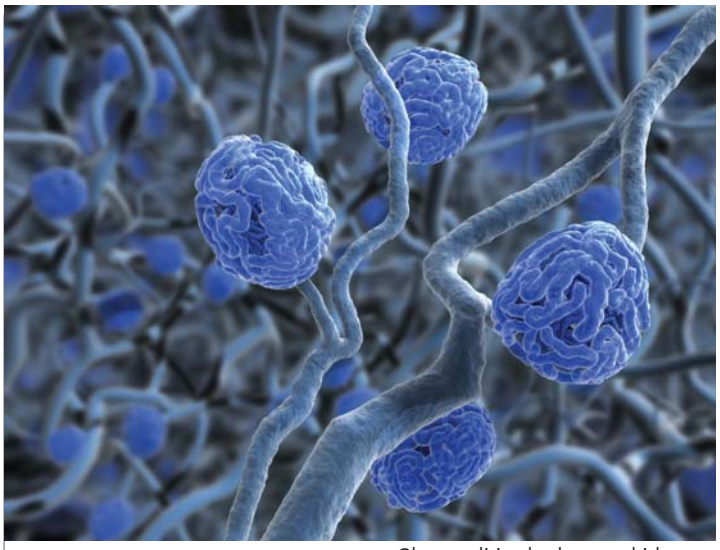
# TOXICOLOGY APPLICATIONS



# Meso Scale Discovery® Toxicology Applications

Preclinical toxicity determination for drug candidates is an area that increasingly requires measurement of several protein biomarkers. The best biomarkers are specific to particular organs or tissue types. Researchers require an assay system with consistent performance, high sensitivity and large dynamic range. MSD's® technology provides all of these characteristics plus multiplexing to save time and precious samples.



Glomeruli in the human kidney

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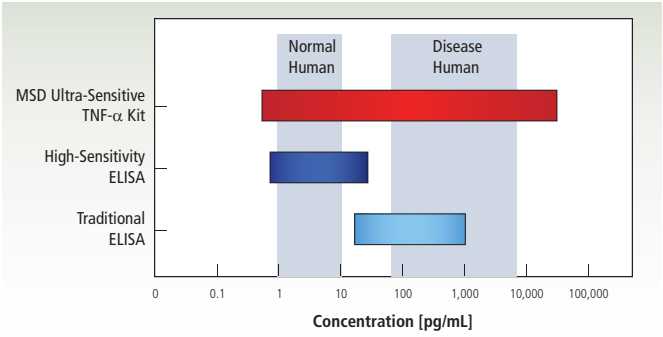
# The MSD Advantage

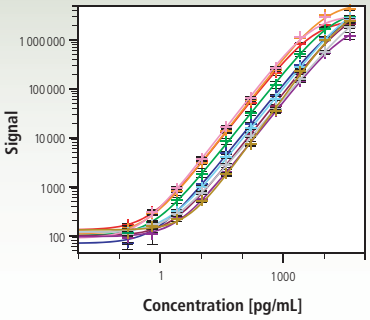
MESO SCALE DISCOVERY'S MULTI-ARRAY® Technology enables the detection of biomarkers in single and multiplex formats. Using the power of our patented electrochemiluminescence detection, MSD offers assays that have ultra-low detection limits, provide up to five logs of linear dynamic range, use minimal sample and handle difficult matrices easily. The extraordinary analytical performance of MSD assays translates into valuable advantages. Our sensitivity and dynamic range enable the development of assays that can measure native levels of biomarkers in normal and diseased samples without multiple dilutions, saving time, resources, sample and cost. Our multiplex panels allow measurements of multiple analytes from a single sample, without extra work or time. Our simple protocols and streamlined formats help validations go smoothly. These advantages—and many others—are why so many people use MSD assays. It's not only what MSD assays can do… it's what you can do with MSD assays.

## Rapid, Robust, Reproducible




- Large dynamic range
- High sensitivity
- High precision
- Low background
- Conserves sample volume
- Simple protocols
- Reduces matrix effects
- Eliminates multiple dilutions

## Multiplexing




- Multiple analytes in one well
- No compromise in performance or speed
- Catalog assay panels for rapid delivery
- Custom panels available

## Assay Solutions and Services

- Over 300 single analyte kits
- Over 200 multiple analyte kits
- Customized multiplexed kits
- Qualified kits
- ELISA conversion packs
- QuickPlex® packs
- Prototype Printing Services
- On-site scientific support
- Contract assay development

## SECTOR® Instruments

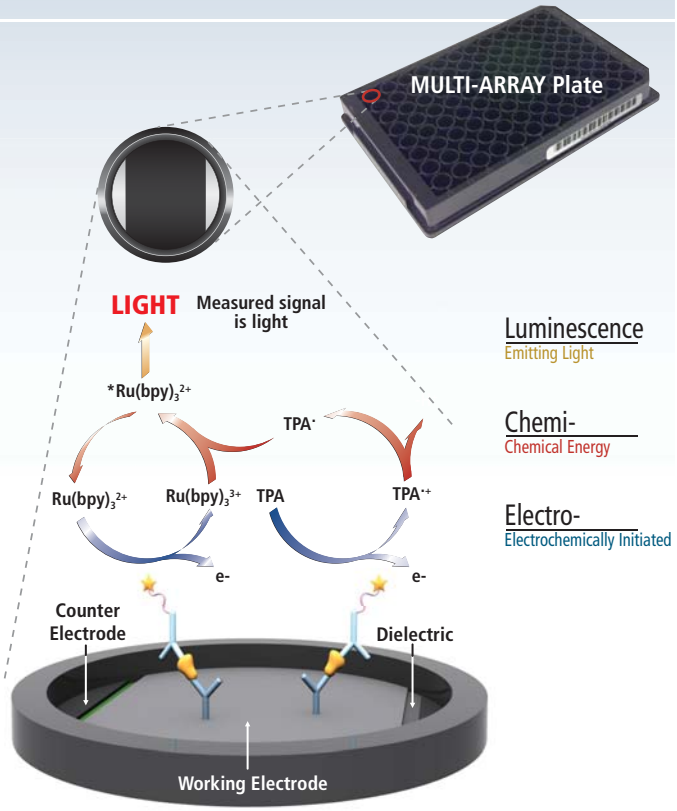
- Ultra-fast read time
- No fluidics
- No calibration required
- Reliable measurements
- Integrated data analysis tool
- Comprehensive validation packages
- 21 CFR 11 compliant software
- Comprehensive service plans
- On-site assistance

# MSD Technology

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.

## Electrochemiluminescence Features:

- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity
- Flexible surface coatings to suit most any biology
- Carbon electrode plate surface has 10X greater binding capacity than polystyrene
- Custom surface coatings and patterns




## MULTI-ARRAY and MULTI-SPOT Features:

- Capability to simultaneously measure multiple analytes in the same well
- High density arrays for high throughput multiplexing of biomarkers
- The unique bar code label on each plate enables complete traceability back to MSD manufacturing records
- The MSD DISCOVERY WORKBENCH® software provides customers with a powerful tool for data analysis


# Nephrotoxicity

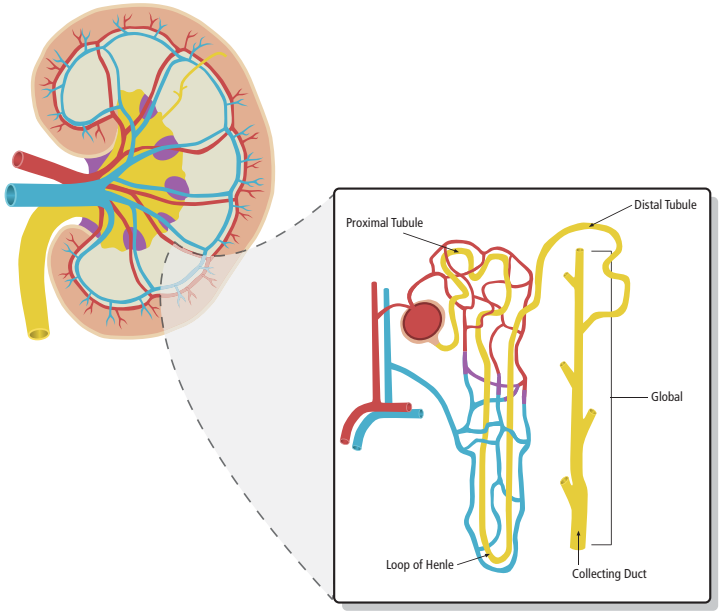
## Representative Nephrotoxicity Products for Toxicology Applications

| | | |
|---|---|---|
| Clusterin (rat)* | NGAL (human)‡ | B2M (rat)† |
| TIM-1/KIM-1/HAVCR (rat)* | VEGF (rat) | IL-18 (human)‡ |
| Argutus AKI Test® (rat)* (αGST, GSTYb1, RPA-1) | Kidney Injury Panel 1 (rat)* (Lipocalin-2, Osteopontin, Albumin, TIM-1) | Kidney Injury Panel 2 (rat)† (Lipocalin-2, Osteopontin, Albumin, TIM-1, αGST, Cystatin C, Clusterin) |

* Qualified Kit † In Development ‡ Prototype

For a complete list of products please check our website at www.mesoscale.com
Please inquire about other human injury markers.

Traditional clinical markers for kidney injury, such as BUN and serum creatinine, are not sensitive enough to detect subtle kidney damage and often do not correlate to damage measured by histopathology. The Food and Drug Administration (FDA) and The European Agency for the Evaluation of Medicinal Products (EMEA) have acknowledged the need for novel biomarkers to identify kidney injury earlier in the drug discovery process. MSD, working with members of the Critical Path Institute (C-Path) and Health and Environmental Sciences Institute (HESI) consortiums, has developed singleplex and multiplex panels to rapidly screen large numbers of compounds for drug induced kidney toxicity. Our Argutus AKI Test, which includes αGST, GSTYb1, and RPA-1, was developed in conjunction with Argutus Medical (formerly Biotrin International). HESI samples tested on this panel have been shown to correlate well to previous measurements. Our Kidney Injury Panel 1 (rat) includes assays for albumin, TIM-1 (a.k.a. KIM-1 or HAVCR), NGAL (a.k.a. Lipocalin-2) and osteopontin. All four analytes have been shown by C-Path Predictive Safety Testing Consortium (PSTC) members to be indicative of early detection of kidney damage. Many of these analytes have been submitted for approval to the FDA as biomarkers for pre-clinical nephrotoxicity measurements. MSD continues to be at the forefront of developing new panels and singleplex assays for toxicity applications.

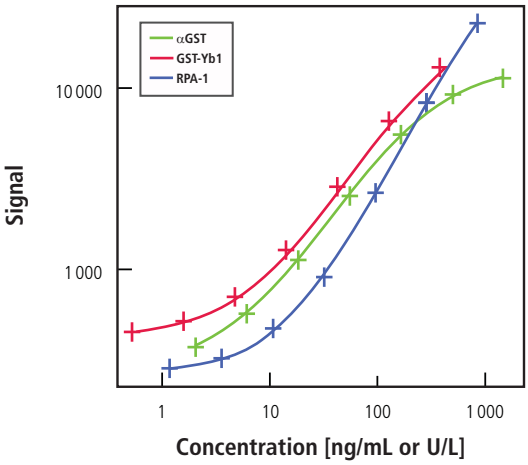



# Nephrotoxicity

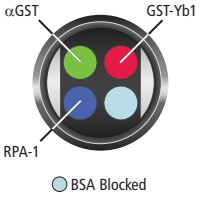
## Argutus AKI Test®

The Argutus AKI (Acute Kidney Injury) Test measures three biomarkers of drug-induced nephrotoxicity: αGST, GST Yb1, and RPA-1. The HESI recently submitted these biomarkers to the FDA as predictive biomarkers. By combining measurements of αGST (proximal tubular injury), GST Yb1 (distal tubular injury) and RPA-1 (collecting duct injury), the specific location of kidney injury can be inferred.[1,2] Collectively, these biomarkers offer a quantitative assessment of the location and extent of acute kidney injury. We qualified this panel according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W., et. al[3]. The qualification procedure involved measurement of control samples on multiple days, establishment of limits of quantitation, spike recovery, dilutional linearity, and measurement of control and treated samples.

### *αGST, GSTYb1, and RPA-1*




| | Analyte | | |
|---|---|---|---|
| | 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 |




LLOD (Lower Limit of Detection) is defined as 2.5 SD (Standard Deviation) over the background signal.

The LLOQ (Lower Limit of Quantitation) 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 (Upper Limit of Quantitation) 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%.

### Precision: Multi-Day Study

Three levels of controls (high, mid, and low) were prepared for precision studies. Controls were made by spiking papilla medulla extract into rat urine.

| | Control | Plates | Concentration | Average Intra-plate % CV | Inter-day % CV |
|---|---|---|---|---|---|
| αGST (ng/mL) | High | 8 | 79.1 | 2.9 | 6.5 |
| | Mid | 8 | 61.7 | 3.3 | 6.6 |
| GSTYb1 (ng/mL) | High | 8 | 86.5 | 4.1 | 5.1 |
| | Low | 8 | 28.4 | 4.3 | 5.7 |
| RPA-1 (U/L) | High | 8 | 471 | 4.7 | 5.6 |
| | Mid | 8 | 89.6 | 5.5 | 6.3 |

### Samples

Rat urine samples from known injury inducing drugs were run on the Argutus AKI Test at 5-fold dilution. 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 | Concentration (ng/mL) | Concentration CV | Concentration (ng/mL) | Concentration CV | Concentration (U/L) | Concentration 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 | Alpha-GST | B680 | 69.2 | 3.9 | *38.0* | *36.6* | 757 | 6.3 |
| | | B681 | 176 | 2.6 | *43.3* | *30.2* | 704 | 4.4 |

### Catalog Numbers for Argutus AKI Test Qualified Kit

| | Kit Size | Catalog Numbers |
|---|---|---|
| Rat | 1 Plate | K15156C-1 |
| | 5 Plate | K15156C-2 |
| | 25 Plate | K15156C-4 |

### Example Protocol

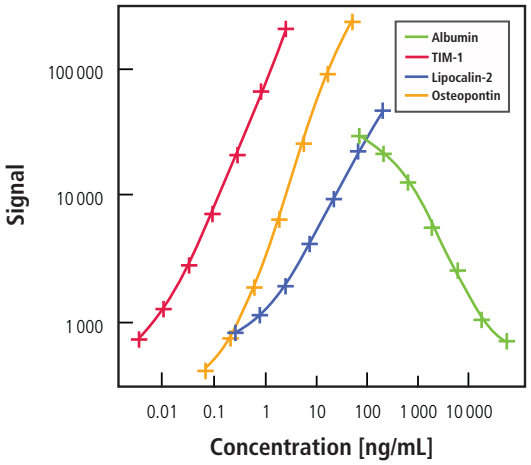
1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 25 µL Diluent, add 25 µL Calibrator/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

# Nephrotoxicity

## Kidney Injury Panel 1 (rat)

Our Kidney Injury Panel 1 (rat) measures albumin, TIM-1, lipocalin-2 (NGAL), and osteopontin (OPN) in urine samples. Albumin is very abundant (10's of µg/mL); we measure it by a competitive immunoassay to enable multiplexing with lower-abundance biomarkers. The other analytes are measured by sandwich immunoassays. Albumin and TIM-1 are now biomarkers that the FDA and EMEA will consider during their drug review process.[4] Lipocalin-2 and osteopontin have also been implicated as important biomarkers of nephrotoxicity and acute kidney injury and have been studied extensively by CPATH members.[5,6,7,8] We qualified this panel according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W., et. al[3]. The qualification procedure involved measurement of control samples on multiple days, establishment of limits of quantitation, spike recovery, dilutional linearity, and measurement of control and treated samples.

### *Albumin, TIM-1, Lipocalin-2, and Osteopontin*

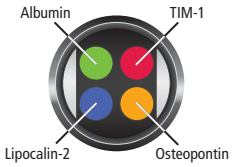



| | Analyte | | |
|---|---|---|---|
| | TIM-1 | Lipocalin-2 | Osteopontin |
| LLOD (ng/mL) | 0.0013 | 0.250 | 0.066 |
| LLOQ (ng/mL) | 0.02 | 1.56 | 0.39 |
| ULOQ (ng/mL) | 1.88 | 150 | 37.5 |

| | Analyte |
|---|---|
| | Albumin |
| LLOD (ng/mL) | 108 |
| LLOQ (ng/mL) | 781 |
| Upper Limit (ng/mL) | 28481 |

LLOD is defined at 80% of the maximum signal for albumin.

The upper limit of albumin was based on 20% of the signal over the highest calibrator. The LLOQ for albumin is the point on the standard curve where the %CV of the standard is less than 20% and the percent recovery of the standard was between 80% and 120%.




### Precision: Multi-Day Study

A multi-day, multi-plate study over 13 plates was performed to show reproducibility. In addition to the standard curves, control samples (high, mid and low) were measured on each plate. Each sample was run in triplicate. The average intra-plate %CV and inter-plate %CV of the concentrations are shown below.

| | Control | Plates | Concentration (ng/mL) | Average Intra-plate % CV | Inter-plate % CV |
|---|---|---|---|---|---|
| Albumin | High | 13 | 12040 | 3.5 | 5.8 |
| | Mid | 13 | 4137 | 3.4 | 4.5 |
| | Low | 13 | 1765 | 3.1 | 7.2 |
| TIM-1 | High | 13 | 0.960 | 3.5 | 4.4 |
| | Mid | 13 | 0.329 | 3.8 | 5.7 |
| | Low | 13 | 0.133 | 4.2 | 9.9 |
| Lipocalin-2 | High | 13 | 194 | 8.4 | 10.4 |
| | Mid | 13 | 91.5 | 6.4 | 9.0 |
| | Low | 13 | 42.3 | 5.6 | 8.2 |
| Osteopontin | High | 13 | 15.6 | 5.0 | 6.4 |
| | Mid | 13 | 6.06 | 4.5 | 6.4 |

### Catalog Numbers for Kidney Injury Panel 1 Qualified Kit

| | Kit Size | Catalog Numbers |
|---|---|---|
| Rat | 1 Plate | K15162C-1 |
| | 5 Plate | K15162C-2 |
| | 25 Plate | K15162C-4 |

### Example Protocol

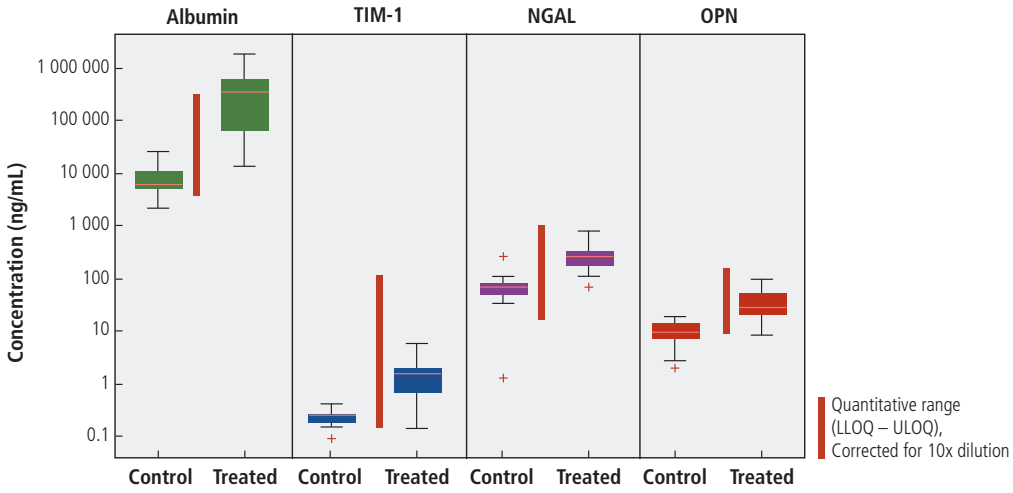
1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 50 µL sample or standard premixed with Albumin tracer, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

# Nephrotoxicity

## Kidney Injury Panel 1 (rat)

### Samples

Rat urine samples were assayed at 10-fold dilution on the Kidney Injury Panel 1. The "treated" samples tested were from rats exposed to known nephrotoxicants prior to sample collection. Some treated samples that assayed above the ULOQ were assayed again at 40-fold dilution. For all of the analytes on the panel, significant correspondence between histo-pathology score and abundance was observed (data not shown).




**Catalog Numbers for Kidney Injury Panel 1 Qualified Kit**

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|---|---|---|
| Rat | 1 Plate | K15162C-1 |
| | 5 Plate | K15162C-2 |
| | 25 Plate | K15162C-4 |

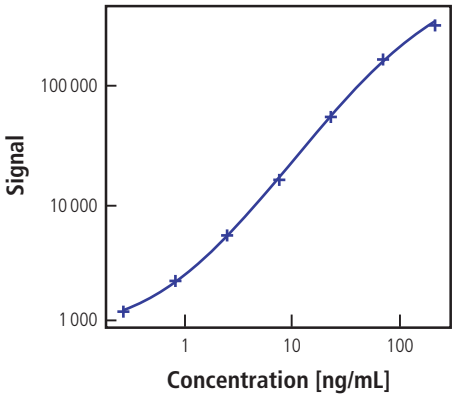
**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 50 µL sample or standard premixed with Albumin tracer, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

## Rat Clusterin

Urinary clusterin has been accepted by the FDA and EMEA as a biomarker to be considered during drug review.[4] The MSD assay for clusterin in rat urine samples is currently available as a singleplex qualified kit that offers excellent sensitivity and dynamic range. We qualified this panel 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 qualification procedure involved measurement of control samples on multiple days, establishment of limits of quantitation, spike recovery, dilutional linearity, and measurement of control and treated samples.

### *Clusterin*




| | Clusterin (ng/mL) |
|---|---|
| LLOD | 0.017 |
| LLOQ | 0.82 |
| ULOQ | 150 |

**Catalog Numbers for Clusterin Qualified Kit**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Rat | 1 Plate | K153HXC-1 |
| | 5 Plate | K153HXC-2 |
| | 25 Plate | K153HXC-4 |

**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 25 µL Assay Diluent, add 25 µL Calibrator/ Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

# Nephrotoxicity

## Rat Clusterin

### Precision: Multi-Day Study

High, mid, and low controls were made by spiking recombinant protein into stock calibrator diluent. The controls were run in quadruplicate on each of 10 plates run across three days. The controls were run at a 10-fold dilution.

| | Control | Plates | Concentration (ng/mL) | Average Intra-plate % CV | Inter-day % CV |
|---|---|---|---|---|---|
| Clusterin | High | 10 | 139 | 7.27 | 10.4 |
| | Mid | 10 | 17.5 | 4.30 | 6.06 |
| | Low | 10 | 1.90 | 4.80 | 6.57 |

## Spike Recovery

Rat urine samples were spiked with calibrators at multiple values throughout the range of the assay. For the Argutus AKI Test panel and the Rat Clusterin assay, the Calibrators were spiked into 5-fold diluted rat urine. For the Kidney Injury Panel 1 panel the spiked samples were tested at a 10-fold dilution into diluent. The recombinant Osteopontin may be bound to proteins in the urine, making it under-recover.

% Recovery = measured / expected x 100

| | % Recovery in Urine Sample | | | |
|---|---|---|---|---|
| Analyte | High Conc. | Mid Conc. | Low Conc. | Kit |
| αGST | 100 | 94 | 105 | Argutus AKI Test |
| GST-Yb1 | 95 | 105 | 94 | Argutus AKI Test |
| RPA-1 | 95 | 102 | 98 | Argutus AKI Test |
| Albumin | 102 | 101 | 98 | Kidney Injury Panel 1 (rat) |
| TIM-1 | 95 | 101 | 104 | Kidney Injury Panel 1 (rat) |
| Lipocalin-2 | 103 | 106 | 99 | Kidney Injury Panel 1 (rat) |
| Osteopontin | 63 | 53 | 38 | Kidney Injury Panel 1 (rat) |
| Clusterin | 94 | 93 | 97 | Rat Clusterin |

## Dilutional Linearity

Serial dilutions of rat urine samples were tested to assess linearity. Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

| | % Dilutional Linearity in Urine Sample | | | | | | |
|---|---|---|---|---|---|---|---|
| Analyte | 5 | 10 | 20 | 40 | 80 | 160 | Kit |
| αGST | 102 | 101 | - | - | - | - | Argutus AKI Test |
| GST-Yb1 | 105 | 84 | 86 | - | - | - | Argutus AKI Test |
| RPA-1 | 86 | 80 | 89 | 96 | 100 | 94 | Argutus AKI Test |
| Albumin | - | 105 | 103 | 100 | - | - | Kidney Injury Panel 1 (rat) |
| TIM-1 | - | 109 | 101 | 98 | - | - | Kidney Injury Panel 1 (rat) |
| Lipocalin-2 | - | 145 | 112 | 103 | - | - | Kidney Injury Panel 1 (rat) |
| Osteopontin | - | 193 | 187 | 179 | - | - | Kidney Injury Panel 1 (rat) |
| Clusterin | - | 125 | 119 | 107 | 106 | - | Rat Clusterin |

# Myotoxicity

**Representative Myotoxicity Products for Toxicology Applications**

Skeletal Troponin I (sTnI) (rat)*

Cardiac Injury Panel 2 (rat)*
(cTnI, cTnT and FABP3)

Cardiac Injury Panel 3 (rat)*
(cTnI, cTnT, FABP3, Myl3)

Muscle Injury Panel 1 (rat)*
(cTnI, cTnT, sTnI, FABP3, Myl3)

Muscle Injury Panel 2 (rat)†
(Paravalbumin, TIMP-1, CK)

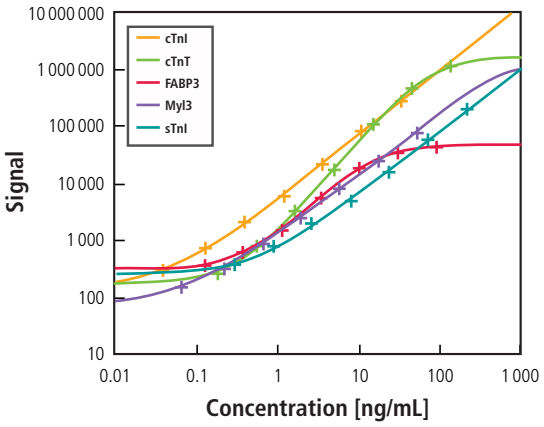
* Qualified Kit † In Development

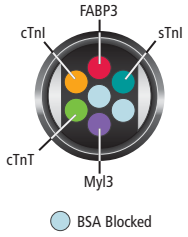
For a complete list of products please check our website at www.mesoscale.com

## Muscle Injury Panel 1 (rat)

Muscle Injury Panel 1 (rat) measures five important biomarkers of drug-induced myotoxicity.[9,10] The biomarkers on the panel were selected for their ability to collectively distinguish myocardial damage from damage to skeletal muscle or other tissues. Using these biomarkers, damage to skeletal muscle can be additionally stratified into slow and fast-twitch types. The selectivity of the assays available from MSD is demonstrated in the specificity section below. We qualified this panel according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W., et al[3]. The qualification procedure involved measurement of control samples on multiple days, establishment of limits of quantitation, spike recovery, dilutional linearity, and measurement of control and treated samples.

### *cTnI, cTnT, FABP3, Myl3, and sTnI*




| | Analyte | | | | |
|---|---|---|---|---|---|
| | cTnI | cTnT | FABP3 | Myl3 | sTnI |
| LLOD (ng/mL) | 0.010 | 0.079 | 0.069 | 0.023 | 0.148 |
| LLOQ (ng/mL) | 0.098 | 0.488 | 0.391 | 0.054 | 0.781 |
| ULOQ (ng/mL) | 20.0 | 100 | 15.0 | 44.0 | 160 |

### Samples

Serum, heparin plasma, and EDTA plasma samples collected from normal Sprague-Dawley rats were tested at 2-fold dilution on the Muscle Injury Panel 1 (rat). Shown here are the median and range of concentrations for each sample set. Skeletal Troponin I was below the quantitative range for all samples.

| Sample | Statistic | cTnI | cTnT | FABP3 | Myl3 | sTnI |
|---|---|---|---|---|---|---|
| Serum | Median (ng/mL) | 1.59 | 0.83 | 19.84 | 0.61 | < 1.56 |
| | Range (ng/mL) | 0.44 - 3.53 | < 0.976 - 2.04 | 5.28 - > 30 | 0.25 - 1.05 | < 1.56 |
| | N | 10 | 10 | 10 | 10 | 10 |
| Heparin Plasma | Median (ng/mL) | 1.81 | 1.06 | 28.35 | 0.55 | < 1.56 |
| | Range (ng/mL) | 0.37 - 4.10 | < 0.976 - 2.79 | 4.12 - > 30 | 0.16 - 1.16 | < 1.56 |
| | N | 10 | 10 | 10 | 10 | 10 |
| EDTA Plasma | Median (ng/mL) | 2.31 | 1.28 | 35.63 | 0.81 | < 1.56 |
| | Range (ng/mL) | 1.45 - 4.05 | < 0.976 - 2.80 | 19.6 - > 30 | 0.43 - 1.17 | < 1.56 |
| | N | 10 | 10 | 10 | 10 | 10 |

### Catalog Numbers for Muscle Injury Panel 1 Qualified Kit

| | Kit Size | Catalog Numbers |
|---|---|---|
| Rat | 1 Plate | K15181C-1 |
| | 5 Plate | K15181C-2 |
| | 25 Plate | K15181C-4 |

### Example Protocol

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 25 µL Antibody Diluent, add 25 µL Calibrator/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

# Myotoxicity

## Muscle Injury Panel 1 (rat)

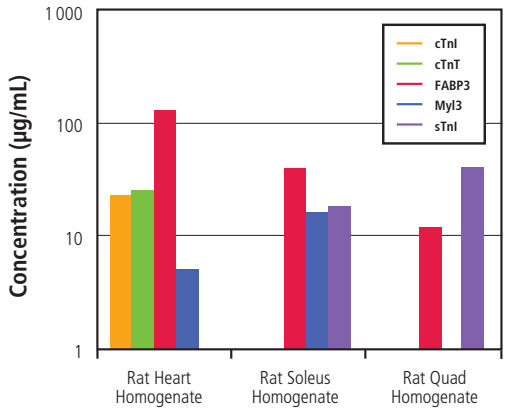
### Precision: Multi-Day Study

Control samples were measured on 9 plates across 3 days. The controls were run in triplicate or quadruplicate on each plate. Normal rat serum, rat soleus homogenate, and assay Calibrators are used to make control samples. The high control contains 25% normal rat serum and Calibrators. The mid control contains rat soleus homogenate and Calibrators. The low control contains only the assay Calibrators. The average intra-plate %CV and inter-plate %CV of the concentrations are shown below.

| | Control | Plates | Concentration (ng/mL) | Average Intra-plate % CV | Inter-plate % CV |
|---|---|---|---|---|---|
| cTnI | High | 9 | 9.36 | 3.8 | 5.9 |
| | Mid | 9 | 1.31 | 3.9 | 6.0 |
| | Low | 9 | 0.28 | 4.0 | 5.1 |
| cTnT | High | 9 | 35.4 | 3.9 | 5.0 |
| | Mid | 9 | 6.46 | 2.5 | 4.0 |
| | Low | 9 | 1.06 | 3.3 | 4.6 |
| FABP3 | High | 9 | 13.9 | 5.9 | 7.5 |
| | Mid | 9 | 8.44 | 5.5 | 7.6 |
| | Low | 9 | 2.85 | 2.8 | 4.8 |
| Myl3 | High | 9 | 30.3 | 5.9 | 7.0 |
| | Mid | 9 | 3.01 | 3.8 | 6.9 |
| | Low | 9 | 0.32 | 6.0 | 7.1 |
| sTnI | High | 9 | 108 | 4.3 | 5.8 |
| | Mid | 9 | 17.4 | 2.4 | 3.1 |
| | Low | 9 | 2.88 | 3.5 | 10.5 |

### Specificity

Tissue homogenates from heart, fast twitch, and slow twitch muscle were tested at 100X, 1000X, and 10000X sample dilution. The assays for cardiac troponins were positive for cardiac homogenates and negative for other muscle homogenates, demonstrating specificity for cardiac tissue. The assay for skeletal Troponin I was specific for fast and slow twitch skeletal muscle. The assay measured FABP3 in cardiac muscle and skeletal muscle. The slow twitch muscle was positive for Myl3, while approximately 200X less Myl3 was measured in fast twitch. Concentrations below are corrected for dilution.




| | cTnI | | cTnT | | FABP3 | | Myl3 | | sTnI | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample Group | Sample Dilution | Conc. (µg/mL) | Sample Dilution | Conc. (µg/mL) | Sample Dilution | Conc. (µg/mL) | Sample Dilution | Conc. (µg/mL) | Sample Dilution | Conc. (µg/mL) |
| Rat Heart Homogenate | 1000 | 22.6 | 1000 | 25.1 | 10000 | 125.2 | 1000 | 5.0 | 100 | < LLOD |
| Rat Soleus Homogenate (slow twitch) | 100 | < LLOD | 100 | < LLOD | 10000 | 38.8 | 1000 | 16.4 | 1000 | 18.1 |
| Rat Quad Homogenate (fast twitch) | 100 | < LLOD | 100 | < LLOD | 1000 | 12.2 | 100 | 0.08 | 1000 | 40.9 |

# Myotoxicity

## Spike Recovery

Rat serum, EDTA plasma and heparin plasma samples were spiked with calibrators at multiple values throughout the range of the assay. For the Rat sTnI assay, the Calibrators were spiked into 2-fold diluted samples. For the Muscle Injury Panel 2 assay, the Calibrators were spiked into 20-fold diluted plasma and 100-fold diluted serum samples.

% Recovery = measured / expected x 100

| | % Recovery | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Serum | | | EDTA Plasma | | | Heparin Plasma | | | |
| Analyte | High Conc. | Mid Conc. | Low Conc. | High Conc. | Mid Conc. | Low Conc. | High Conc. | Mid Conc. | Low Conc. | Kit |
| cTnI | 100 | 106 | 105 | 96 | 111 | 97 | 103 | 104 | 93 | Cardiac Injury Panel 3 (rat) |
| | 116 | 116 | 111 | 128 | 116 | 108 | 134 | 123 | 105 | Muscle Injury Panel 1 (rat) |
| cTnT | 88 | 89 | 89 | 83 | 90 | 99 | 113 | 112 | 106 | Cardiac Injury Panel 3 (rat) |
| | 77 | 86 | 86 | 89 | 78 | 71 | 100 | 104 | 95 | Muscle Injury Panel 1 (rat) |
| FABP3 | 108 | 96 | 105 | 100 | 108 | 104 | 103 | 97 | 115 | Cardiac Injury Panel 3 (rat) |
| | 114 | 113 | 98 | 100 | 93 | 91 | 90 | 99 | 87 | Muscle Injury Panel 1 (rat) |
| Myl3 | 110 | 106 | 108 | 120 | 114 | 104 | 112 | 105 | 96 | Cardiac Injury Panel 3 (rat) |
| | 115 | 120 | 105 | 136 | 131 | 107 | 138 | 128 | 107 | Muscle Injury Panel 1 (rat) |
| sTnI | 108 | 106 | 106 | 100 | 96 | 91 | 109 | 101 | 96 | Rat Skeletal Troponin I (sTnI) |
| | 101 | 109 | 100 | 107 | 108 | 99 | 110 | 107 | 100 | Muscle Injury Panel 1 (rat) |
| Parvalbumin | 119 | 123 | 120 | 122 | 114 | 112 | 136 | 121 | 117 | Muscle Injury Panel 2 (rat) |
| TIMP-1 | 111 | 97 | 90 | 123 | 105 | 101 | 123 | 101 | 97 | Muscle Injury Panel 2 (rat) |
| CK | 93 | 107 | 123 | 96 | 108 | 107 | 92 | 99 | 105 | Muscle Injury Panel 2 (rat) |

## Dilutional Linearity

Serial dilutions of rat serum, EDTA plasma and heparin plasma samples were tested to assess linearity. For Cardiac Injury Panel 3 markers iso-proterenol treated rat serum was used. Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

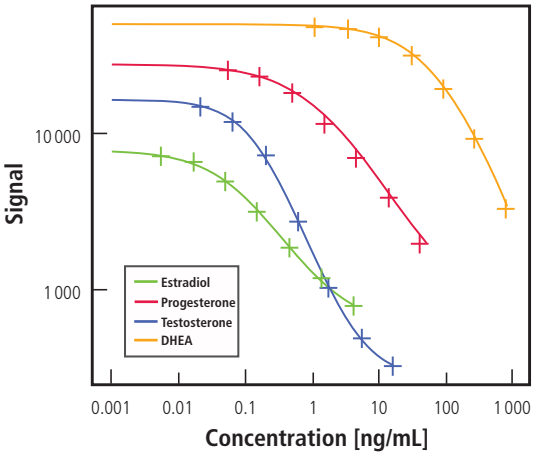
| | % Dilutional Linearity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Serum | | | EDTA Plasma | | | Heparin Plasma | | | |
| Analyte | 2-fold | 4-fold | 8-fold | 2-fold | 4-fold | 8-fold | 2-fold | 4-fold | 8-fold | Kit |
| cTnI | 82 | 84 | 87 | 90 | 91 | 93 | 97 | 90 | 94 | Cardiac Injury Panel 3 (rat) |
| | 101 | 90 | 92 | 108 | 109 | <LLOD | 98 | 96 | 89 | Muscle Injury Panel 1(rat) |
| cTnT | 99 | 83 | 77 | 104 | 88 | <LLOD | 92 | 93 | <LLOQ | Cardiac Injury Panel 3 (rat) |
| | <LLOD | <LLOD | <LLOD | <LLOD | <LLOD | <LLOD | 101 | <LLOD | <LLOD | Muscle Injury Panel 1(rat) |
| FABP3 | >ULOQ | NA | 107 | >ULOQ | NA | 81 | >ULOQ | NA | 85 | Cardiac Injury Panel 3 (rat) |
| | NA | 77 | 88 | 93 | 100 | <LLOD | NA | 93 | 104 | Muscle Injury Panel 1(rat) |
| Myl3 | 91 | 86 | 82 | 82 | 83 | 91 | 99 | 91 | 90 | Cardiac Injury Panel 3 (rat) |
| | 86 | 92 | 96 | 99 | 94 | 89 | 102 | 96 | 107 | Muscle Injury Panel 1(rat) |
| sTnI | 79 | 87 | 92 | 104 | 91 | 92 | 88 | 95 | 97 | Muscle Injury Panel 1(rat) |
| | 20-fold | 40-fold | 80-fold | 20-fold | 40-fold | 80-fold | 20-fold | 40-fold | 80-fold | |
| Parvalbumin | - | - | - | 75 | 82 | 97 | - | - | - | Muscle Injury Panel 2 (rat) |
| TIMP-1 | - | - | - | 102 | 87 | <LLOQ | - | - | - | Muscle Injury Panel 2 (rat) |
| CK | - | - | - | 82 | 110 | <LLOQ | - | - | - | Muscle Injury Panel 2 (rat) |

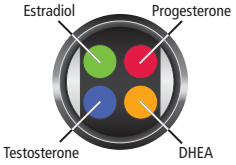
# Reproductive Toxicity

## Steroid Hormone Panel 1 (human, mouse, rat)

Toxicity to the endocrine system is a common mode of failure for drugs in the clinic, and steroid hormones are recognized as important biomarkers in this area. The Steroid Hormone Panel 1 is designed to measure critical steroid hormones in serum and plasma from human, mouse, and rat.

### *Estradiol, Progesterone, Testosterone and DHEA*




| | Analyte | | | |
|---|---|---|---|---|
| | Estradiol (ng/mL) | Progesterone (ng/mL) | Testosterone (ng/mL) | DHEA (ng/mL) |
| LLOD | 0.023 | 0.306 | 0.046 | 13.7 |
| LLOQ | 0.049 | 0.617 | 0.198 | 29.6 |
| ULOQ | 4.0 | 50.0 | 5.33 | 800 |

### Catalog Numbers for Steroid Hormone Panel 1

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human/ Mouse/ Rat | 1 Plate | K15178C-1 |
| | 5 Plate | K15178C-2 |
| | 25 Plate | K15178C-4 |

### Example Protocol

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash, add 50 µL standard/sample, incubate 2 hours at RT.
3. Add 15 µL tracer, incubate 30 min at RT.
4. Wash, add 150 µL Read Buffer T, read.

# Inflammation and Growth Factors

### Representative Inflammation and Growth Factor Markers

E-Cadherin (human)
P-Cadherin (human)
c-Kit (human)
CRP (human)
bFGF (human)
sFlt-1 (sVEGFR1) (human)
HGF (human)
sICAM-1 (human)
sICAM-3 (human)
KDR (sVEGFR2) (human)
PlGF (human)
SAA (human)
E-Selectin (human)
P-Selectin (human)
TGF-β1 (human)
Thrombomodulin (human)
sVCAM-1 (human)
VEGF (human, mouse/rat)

#### Multiplex Panels

Vascular Injury Panel I (human)
(sICAM-3, E-Selectin, P-Selectin, Thrombomodulin)
Vascular Injury Panel II (human)
(CRP, sICAM-1, sVCAM-1, SAA)
c-Kit (pY721), Total c-Kit
Inflammation Panel 1 (rat)*
(Lipocalin-2, TSP-1, TIMP-1, MCP-1)
Growth Factor Panel I (human)
(bFGF, VEGF, sFlt-1, PlGF)
Growth Factor Panel II (human)
(c-Kit, KDR)
Acute Phase Protein Panel 1 (rat)*
(A2M, AGP)

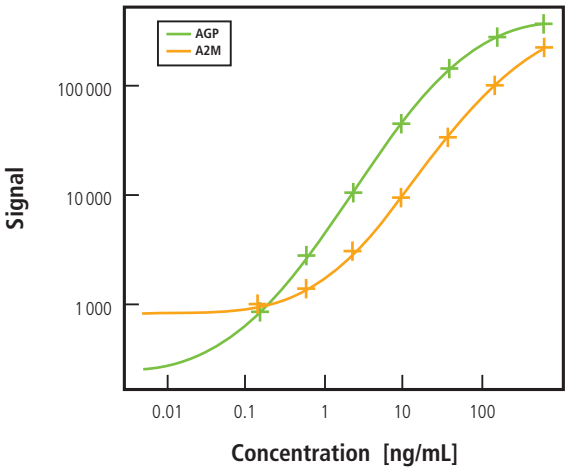
* Qualified Kit

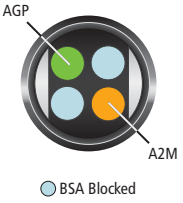
For a complete list of products please check our website at www.mesoscale.com

Circulating levels of cytokines and other inflammation markers have been studied in patients with chronic renal failure[11] and persistent inflammation has been the suspect of other pathophysiologic alterations in chronic kidney disease.[12] Biomarkers of inflammation have also emerged as potential preclinical indicators of heart failure.[13]

## Acute Phase Protein Panel 1 (rat)

### *AGP and A2M*




| | Analyte | |
|---|---|---|
| | AGP (ng/mL) | A2M (ng/mL) |
| LLOD | 0.012 | 0.036 |
| LLOQ | 0.146 | 1.00 |
| ULOQ | 150 | 400 |

### Precision: Multi-Day Study

High, mid, and low controls were defined that are rat serum samples diluted 10000-fold into assay diluent. Samples were chosen that span a large proportion of the dynamic range for each assay. These controls were run in triplicate on each of 9 plates run across 3 days of testing. The concentration values shown below are corrected for the 10000-fold dilution.

| | Control | Plates | Concentration (ng/mL) | Average Intra-plate % CV | Inter-plate % CV |
|---|---|---|---|---|---|
| AGP | High | 9 | 42706 | 5.3 | 9.9 |
| | Mid | 9 | 8427 | 4.2 | 10.1 |
| | Low | 9 | 408 | 3.4 | 7.1 |
| A2M | High | 9 | 215203 | 6.2 | 8.6 |
| | Mid | 9 | 20468 | 2.4 | 6.3 |
| | Low | 9 | 1870 | 3.1 | 4.0 |

### Catalog Numbers for Acute Phase Protein Panel 1 (rat) Qualified Kit

| | Kit Size | Catalog Numbers |
|---|---|---|
| Rat | 1 Plate | K15175C-1 |
| | 5 Plate | K15175C-2 |
| | 25 Plate | K15175C-4 |

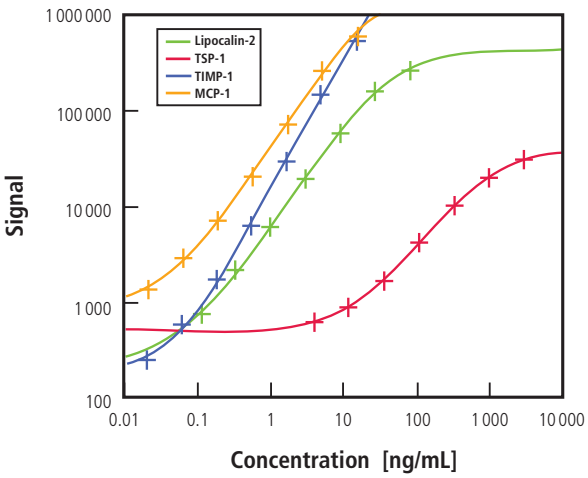
### Example Protocol

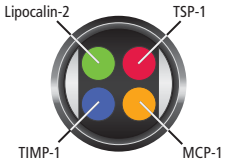
1. Add 25 µL Assay Diluent, incubate 30 min at RT.
2. Add 25 µL of Calibrators/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer, read.

# Inflammation and Growth Factors

## Rat Inflammation Panel 1

### *Lipocalin-2, TSP-1, TIMP-1, and MCP-1*




| | Analyte | | | |
|---|---|---|---|---|
| | Lipocalin-2 (ng/mL) | TSP-1 (ng/mL) | TIMP-1 (ng/mL) | MCP-1 (ng/mL) |
| LLOD | 0.0050 | 3.7 | 0.0046 | 0.0028 |
| LLOQ | 0.050 | 35 | 0.10 | 0.050 |
| ULOQ | 25 | 1500 | 12 | 12 |

### Precision: Multi-Day Study

Control samples of high, mid, and low levels of each analyte were measured on each plate. The controls were run in quadruplicate on each of 21 plates run across multiple days (n>3).

| | Control | Plates | Concentration (ng/mL) | Average Intra-plate % CV | Inter-plate % CV |
|---|---|---|---|---|---|
| Lipocalin-2 | High | 21 | 12.8 | 3.7 | 6.0 |
| | Mid | 21 | 1.58 | 3.5 | 6.9 |
| | Low | 21 | 0.364 | 3.4 | 7.5 |
| TSP-1 | High | 21 | 1083 | 9.8 | 12.5 |
| | Mid | 21 | 658 | 6.4 | 10.6 |
| | Low | 21 | 73.8 | 3.3 | 7.0 |
| TIMP-1 | High | 21 | 6.55 | 6.8 | 9.0 |
| | Mid | 21 | 0.914 | 3.1 | 6.5 |
| | Low | 21 | 0.404 | 4.0 | 6.5 |
| MCP-1 | High | 21 | 9.48 | 4.1 | 6.8 |
| | Mid | 21 | 0.836 | 5.8 | 8.7 |
| | Low | 21 | 0.0945 | 6.2 | 10.7 |

### Example Protocol

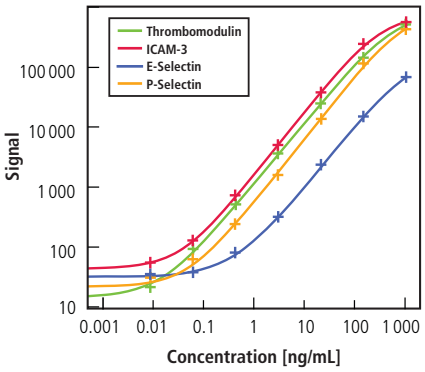
1. Add 25 µL Diluent, incubate 30 minutes at RT.
2. Add 25 µL Calibrator/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

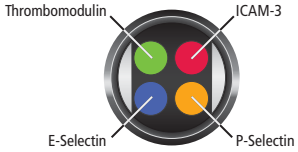
# Inflammation and Growth Factors

## Vascular Injury Panel I (human)

In pre-clinical safety studies, drug-induced vascular injury is of great concern. Endothelial cell dysfunction is considered one of the early indicators of vascular disorders. Drugs may trigger inflammatory signaling cascades and the upregulation of soluble adhesion molecules by activated endothelial cells. Since vascular injury involves multiple mediators and cell types, a multiplex panel of validated biomarkers may serve as a useful predictor of activation of inflammatory cascades in the development of vascular injury.

### *Thrombomodulin, ICAM-3, E-Selectin, and P-Selectin*




| | Analyte | |
|---|---|---|
| | Thrombomodulin | ICAM-3 |
| LLOD (ng/mL) | 0.017 | 0.066 |

| | Analyte | |
|---|---|---|
| | E-Selectin | P-Selectin |
| LLOD (ng/mL) | 0.34 | 0.097 |

Spike Recovery

| | % Recovery | | | |
|---|---|---|---|---|
| | Thrombomodulin | ICAM-3 | E-Selectin | P-Selectin |
| Serum | 87 | 77 | 97 | 89 |
| EDTA Plasma | 69 | 71 | 69 | 85 |
| Heparin Plasma | 83 | 76 | 91 | 91 |

Pooled plasma and serum samples were spiked with known quantities of Thrombomodulin, ICAM-3, E-selectin, P-selectin at different levels and measured using MSD Vascular Injury Panel I.

**Catalog Numbers for Human Vascular Injury I**

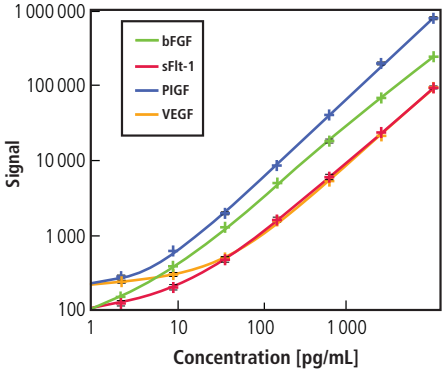
| | Kit Size | Catalog Numbers |
|---|---|---|
| Human | 1 Plate | K15135C-1 |
| | 5 Plate | K15135C-2 |
| | 20 Plate | K15135C-3 |

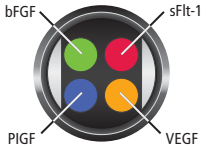
**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 40 µL Assay Diluent, add 10 µL Calibrators/ Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 hour at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer T, read.

## Growth Factor Panel I (human)

### *bFGF, sFlt-1, PIGF, and VEGF*




| | Analyte | | | |
|---|---|---|---|---|
| | bFGF | sFlt-1 | PIGF | VEGF |
| LLOD (pg/mL) | 2.2 | 3.7 | 0.96 | 6.4 |

Spike Recovery

| | % Recovery | | | |
|---|---|---|---|---|
| | bFGF | sFlt-1 | PIGF | VEGF |
| Serum | 99 | 105 | 111 | 114 |
| EDTA Plasma | 77 | 94 | 98 | 95 |

Pooled plasma and serum samples were spiked with known quantities of bFGF, sFlt-1, PIGF, VEGF at different levels and measured using MSD Growth Factor Panel I.

**Catalog Numbers for Human Growth Factor I**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human | 1 Plate | K15029C-1 |
| | 5 Plate | K15029C-2 |
| | 20 Plate | K15029C-3 |

**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 25 µL Assay Diluent, add 25 µL Calibrators/ Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer T, read.

# Cytokines

## Representative Cytokine Markers

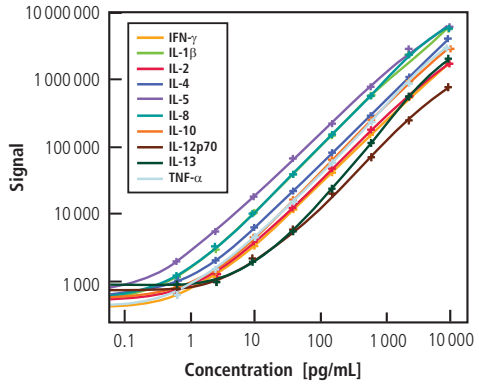
CINC-2 (rat)‡
CINC-3 (rat)‡
CTACK (human)‡
ENA-78 (human)‡
Eotaxin (human)
Eotaxin-3 (human)
G-CSF (human)
GM-CSF (human, mouse, rat, canine)
GRO-α (human)
IFN-α (human)‡
IFN-β (human)
IFN-γ (human, mouse, rat, canine‡)
IL-1α (human, rat)
IL-1β (human, mouse, rat)
IL-1ra (human)‡
IL-2 (human, mouse, rat‡, canine)
IL-4 (human, mouse, rat)
IL-5 (human, mouse, rat)
IL-6 (human, mouse, rat, canine)
IL-6R (human)
IL-7 (human)
IL-8 (human, canine)
IL-10 (human, mouse, rat‡, canine‡)
IL-12 (human, mouse)
IL-12p40 (human, mouse)
IL-12p70 (human, mouse)
IL-13 (human, mouse, rat)
IL-15 (human)
IL-16 (human)
IL-17 (human, mouse)
IL-18 (human)
IL-23 (human)‡
IP-10 (human, mouse)
I-TAC (human)
KC/GRO/CINC (CXCL1) (mouse, rat)
M-CSF (human, mouse‡)
MCP-1 (human, mouse, rat, canine‡)
MCP-2 (human)‡
MCP-4 (human)
MDC (human)
MIG (human)
MIP-1α (human, mouse‡)
MIP-1β (human, mouse‡)
MIP-2 (mouse)‡
MIP-3α (human, mouse, rat)
MIP-3β (human)‡
MMP-1 (human)
MMP-2 (human)
MMP-3 (human)
MMP-8 (human)‡
MMP-9 (human, mouse‡)
MMP-10 (human)
NGAL (human)‡
RANTES (human, mouse)
SDF-1α (human)‡
TARC (human)
TGF-β1 (human)
TIMP-1 (human, rat‡)
TIMP-2 (human)‡
TNF-α (human, mouse, rat, canine)
TNF-RI (human, mouse)
TNF-RII (human, mouse)
TSLP (human)‡
TWEAK (human)‡

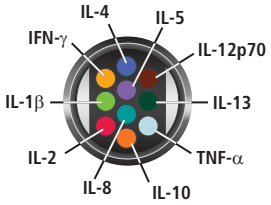
‡ Available through our Prototype Printing Services.

For a complete list of products please check our website at www.mesoscale.com

Multiplexed assays from MSD enable the detection of up to ten cytokines in a single well, in the same amount of time as a singleplex assay—using the same amount of sample!

### *Human TH1/TH2 10-Plex Ultra-Sensitive*




| | Analyte | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | IFN-γ | IL-1β | IL-2 | IL-4 | IL-5 | IL-8 | IL-10 | IL-12p70 | IL-13 | TNF-α |
| LLOD (pg/mL) | 0.39 | 0.18 | 0.67 | 0.31 | 0.076 | 0.14 | 0.36 | 2.3 | 1.8 | 0.48 |

### Catalog Numbers for Human TH1 / TH2 10-Plex Ultra-Sensitive Φ

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human | 1 Plate | K15010C-1 |
| | 5 Plate | K15010C-2 |
| | 25 Plate | K15010C-4 |

Φ Visit www.mesoscale.com for Tissue Culture version of Human TH1 / TH2 10-Plex Kit.

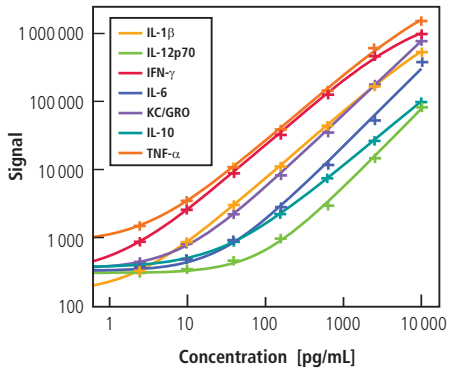
### Example Protocol

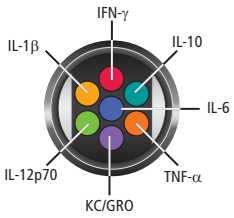
1. Add 25 µL of Assay Diluent to plate, incubate 30 minutes.
2. Add 25 µL of Calibrators/Sample, incubate 2 hours.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 hour.
4. Wash with PBS-T. Add 150 µL Read Buffer T, Read.

# Cytokines

MSD offers several rodent cytokine assays in singleplex and multiplex formats. These assays enable the detection of up to 10 analytes in the same well. The wide dynamic range of the assays enables the measurement of native and disease state levels of cytokines in a single run. The panel on this page shows the sensitivity and range of one of our rodent cytokine assays. The panel was assayed with less than 25 µL of sample.

### *Mouse ProInflammatory 7-Plex Ultra-Sensitive*




| | Analyte | | | | | | |
|---|---|---|---|---|---|---|---|
| | IL-1β | IL-12p70 | IFN-γ | IL-6 | KC/GRO | IL-10 | TNF-α |
| LLOD (pg/mL) | 0.75 | 35 | 0.38 | 4.5 | 3.3 | 11 | 0.85 |

**Catalog Numbers for Mouse ProInflammatory 7-Plex Ultra-Sensitive Φ**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Mouse | 1 Plate | K15012C-1 |
| | 5 Plate | K15012C-2 |
| | 25 Plate | K15012C-4 |

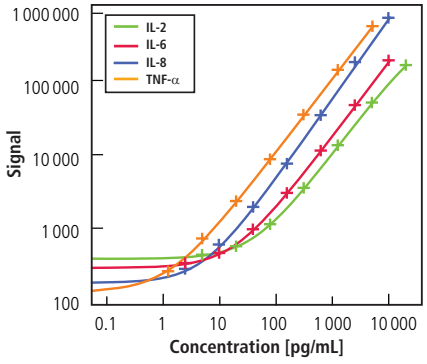
Φ Visit www.mesoscale.com for Tissue Culture version of Mouse ProInflammatory 7-Plex Kit.

**Example Protocol**

1. Add 25 µL Assay Diluent, incubate 30 min at RT.
2. Add 10 to 25 µL of Calibrator/ Sample incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 1 to 2 hours at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer, read.

The exploration of the canine immune system has been driven by the dog's importance as an animal model for understanding the pathogenesis of several canine and human diseases. Dogs are used for drug toxicity trials, transplantation studies and in research of a variety of human diseases such as rheumatoid arthritis, cyclic neutropenia and systemic lupus erythematosus. The Canine ProInflammatory Panel 3 (IL-2, IL-6, IL-8 and TNF-α) is a tool for researchers using canine models to study diseases, drug toxicity as well as vaccine development.

### *Canine ProInflammatory Panel 3 Ultra-Sensitive*





| | Analyte | | | |
|---|---|---|---|---|
| | IL-2 | IL-6 | IL-8 | TNF-α |
| LLOD (pg/mL) | 7.6 | 2.4 | 1.3 | 0.17 |

**Catalog Numbers for Canine ProInflammatory Panel 3 Ultra-Sensitive**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Canine | 1 Plate | K15035C-1 |
| | 5 Plate | K15035C-2 |
| | 25 Plate | K15035C-4 |

**Example Protocol**

1. Add 25 µL Assay Diluent, incubate 30 min at RT.
2. Add 25 µL of Calibrators/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL of Detection Antibody, incubate 2 hours at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer, read.

# Intracellular Signaling

## Representative Intracellular Signaling Markers

Akt (pS473) (human, mouse, rat)
Akt (pT308) (human, mouse)
Akt (Total) (human, mouse, rat)
GSK-3α (pS21) (human)
GSK-3β (pS9) (human)
GSK-3β (Total) (human, mouse, rat)
IRS-1 (pS312) (human)
p70S6K (pT389) (human, mouse, rat)
p70S6K (pT421/pS424) (human, mouse, rat)
p70S6K (Total) (human, mouse, rat)
S6RP (pS235/236) (human, mouse, rat)
S6RP (pS240/244) (human, mouse, rat)
S6RP (Total) (human, mouse, rat)

### Multiplex Panels

Akt (pS473), Total Akt (human, mouse, rat)
GSK-3β (pS9), Total GSK-3β (human)
p70S6K (pT421/pS424), Total p70S6K (human, mouse, rat)
Akt Signaling Panel (Phospho Protein) (pAkt, pp70S6K, pGSK-3β) (human, mouse, rat)
Akt Signaling Panel (Total Protein) (Akt, p70S6K, GSK-3β) (human, mouse, rat)
Insulin Signaling Panel (Phospho Protein) (pIR, pIRS-1, pIGF-1R) (human)
Insulin Signaling Panel (Total Protein) (IR, IRS-1, IGF-1R) (human)

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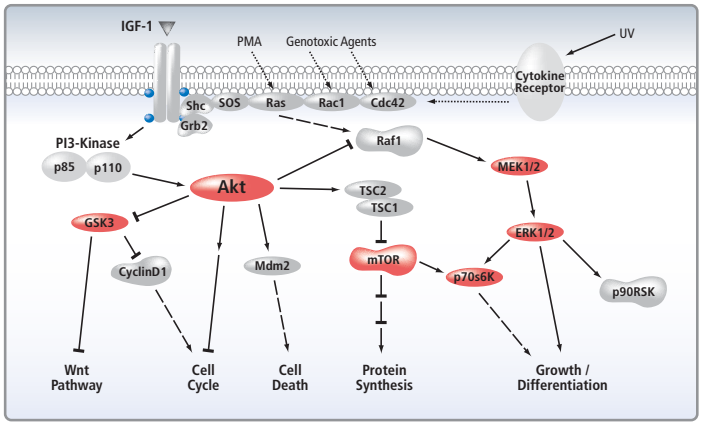
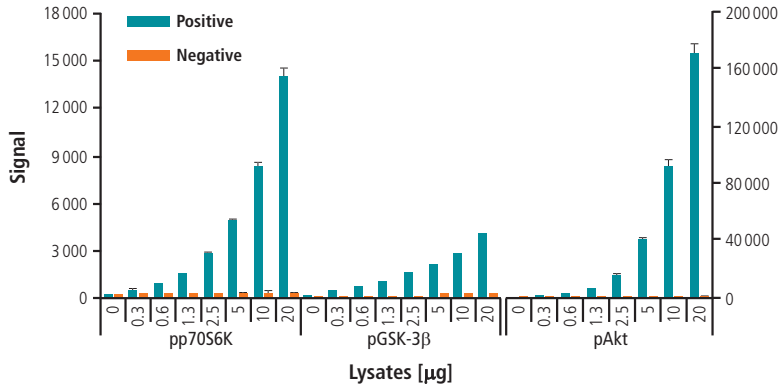
## Cell Signaling

Insulin modulates blood glucose levels by driving glucose uptake and glycogen synthesis. Insulin signaling activates PI3 kinase which results in phosphorylation of Akt. This inhibits downstream substrates including GSK-3β thereby promoting protein synthesis. Akt also activates mTOR which stimulates protein synthesis through p70S6K. In addition, downstream targets of Akt coordinate glycogen synthesis, fatty acid oxidation and protein synthesis.



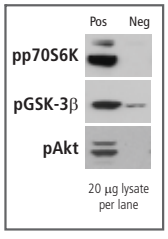

Available AKT/mTOR pathway phosphoprotein targets

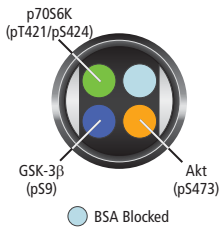
### *Akt Signaling Panel*
### *Akt (pS473), GSK-3β (pS9) and p70S6K (pT421/pS424)*




Logarithmically growing Jurkat cells were treated with PMA (200 nM, 15 min) (positive) or LY294002 (50 µM) and staurosporine (1 µM; 2.25 hr) (negative). Whole cell lysates were added to MSD MULTI-SPOT 96-Well 4 Spot plates coated with analyte-specific capture antibodies on spatially distinct electrodes in each well. Phosphorylated Akt, p70S6K, and GSK-3β were detected with multiplexed sandwich immunoassays using an anti-phospho-specific antibody as the capture or detection antibody in each sandwich.

Traditional Western




### Catalog Numbers for Akt Signaling Panel

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human/ Mouse/ Rat | 1 Plate | K15115D-1 |
| | 5 Plate | K15115D-2 |
| | 20 Plate | K15115D-3 |

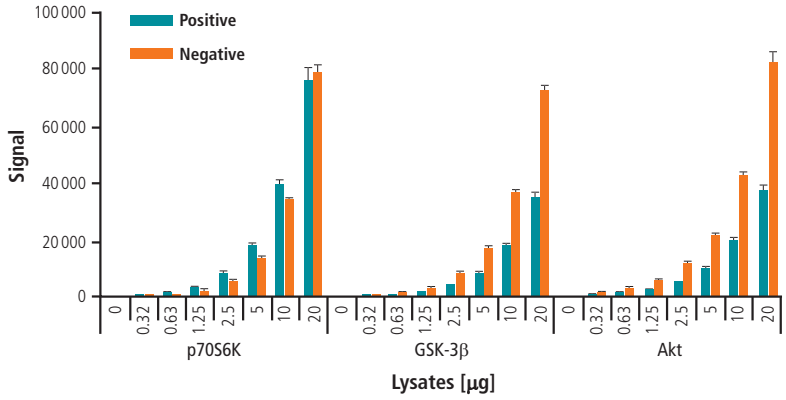
### Example Protocol

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with TBS-T. Add 25 µL lysates, incubate 1 hour at RT.
3. Wash with TBS-T. Add 25 µL Detection Antibodies, incubate 1 hour at RT.
4. Wash with TBS-T. Add 150 µL Read Buffer T, read.

# Intracellular Signaling

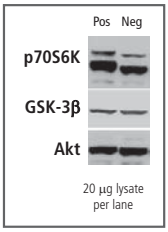
## Cell Signaling

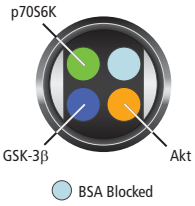
### *Akt Signaling (Total Protein) Panel Akt, GSK-3β and p70S6K*




Logarithmically growing Jurkat cells were treated with PMA (200 nM, 15 min) (positive) or LY294002 (50 µM) and staurosporine (1 µM; 2.25 hr) (negative). Whole cell lysates were added to MSD MULTI-SPOT 96-Well 4 Spot plates coated with analyte-specific capture antibodies on spatially distinct electrodes in each well. Akt, p70S6K, and GSK-3β were detected in multiplex with anti-total-Akt, anti-total-p70S6K, and anti-total-GSK-3β antibodies. Detection antibodies for total proteins recognize epitopes distinct from the capture antibodies.

Traditional Western




BSA Blocked

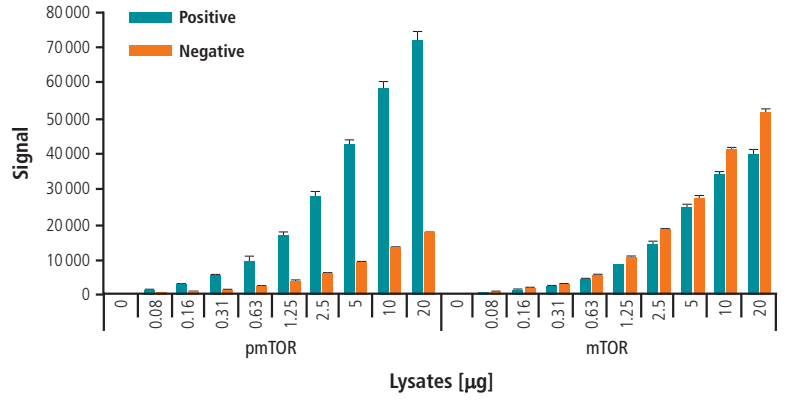
**Catalog Numbers for Akt Signaling Panel (Total Protein)**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human/ Mouse/ Rat | 1 Plate | K15133D-1 |
| | 5 Plate | K15133D-2 |
| | 20 Plate | K15133D-3 |

**Example Protocol**

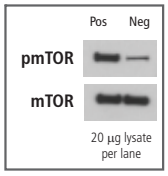
1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with TBS-T. Add 25 µL lysates, incubate 1 hour at RT.
3. Wash with TBS-T. Add 25 µL Detection Antibodies, incubate 1 hour at RT.
4. Wash with TBS-T. Add 150 µL Read Buffer T, read.

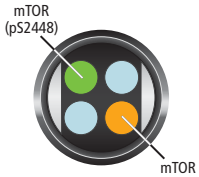
### *Phospho/Total mTOR*




Growing HEK293 cells were treated with Wortmannin (100 nM, 3 h)(negative) or PMA (1 µM, 30 min)(positive). Whole cell lysates were added to MSD MULTI-SPOT 4 Spot plates coated with anti-phospho-mTOR antibody and anti-total-mTOR antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total mTOR were detected with anti-total-mTOR antibody labeled with MSD SULFO-TAG reagent.

Traditional Western




BSA Blocked

**Catalog Numbers for Phospho/Total mTOR**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Human/ Mouse/ Rat | 1 Plate | K15170D-1 |
| | 5 Plate | K15170D-2 |
| | 20 Plate | K15170D-3 |

**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with TBS-T. Add 25 µL lysates, incubate 3 hours at RT.
3. Wash with TBS-T. Add 25 µL Detection Antibody, incubate 1 hour at RT.
4. Wash with TBS-T. Add 150 µL Read Buffer T, read.

# Metabolic Disease

**Representative Metabolic Markers**

Adiponectin (human, mouse, rat)
Ghrelin (mouse/rat)
Active GLP-1 (7-36)amide (human, mouse/rat)
Active GLP-1 (human, mouse/rat)
GLP-1 (7-36)amide (human, mouse/rat)
Total GLP-1 (human, mouse/rat)
Glucagon (human, mouse/rat)
Insulin (human, mouse/rat)
Leptin (human, mouse, rat)
Resistin (human, mouse/rat)

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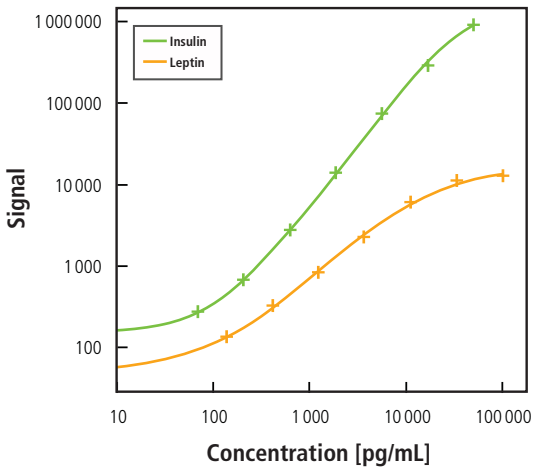
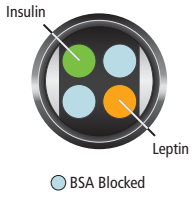
Funicello, et al.[14] used the MSD multiplex Mouse Metabolic Panel to determine simultaneously plasma level of mouse insulin and leptin when studying the implications of proteases in adipogenesis.

## Mouse Metabolic Panel

### *Mouse Insulin and Leptin*




| | Analyte | |
|---|---|---|
| | Insulin | Leptin |
| LLOD (pg/mL) | 49 | 32 |

**Catalog Numbers for Insulin and Leptin**

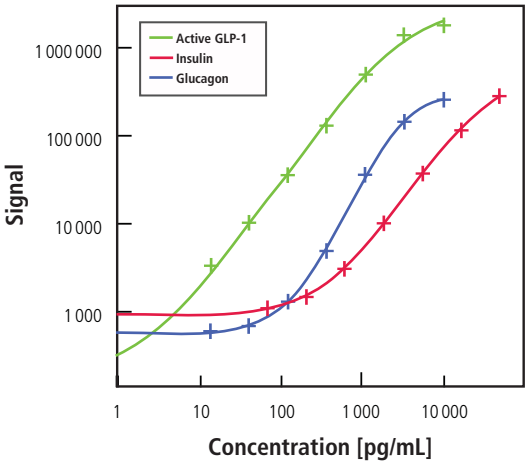
| | Kit Size | Catalog Numbers |
|---|---|---|
| Human | 1 Plate | K15164C-1 |
| | 5 Plate | K15164C-2 |
| | 20 Plate | K15164C-3 |
| Mouse | 1 Plate | K15124C-1 |
| | 5 Plate | K15124C-2 |
| | 20 Plate | K15124C-3 |
| Rat | 1 Plate | K15158C-1 |
| | 5 Plate | K15158C-2 |
| | 20 Plate | K15158C-3 |

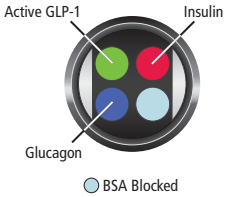
**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 40 µL Detection Antibodies, add 10 µL Calibrator/Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 150 µL Read Buffer T, read.

## Metabolic Multiplex

### *Mouse/Rat Active GLP-1, Insulin, and Glucagon*




| | Analyte | | |
|---|---|---|---|
| | Active GLP-1 | Insulin | Glucagon |
| LLOD (pg/mL) | 0.20 | 53 | 38 |

**Catalog Numbers for Active GLP-1, Insulin, and Glucagon**

| | Kit Size | Catalog Numbers |
|---|---|---|
| Mouse/ Rat | 1 Plate | K15184C-1 |
| | 5 Plate | K15184C-2 |
| | 20 Plate | K15184C-3 |

**Example Protocol**

1. Add 150 µL Blocking Solution, incubate 1 hour at RT.
2. Wash with PBS-T. Add 20 µL Diluent, add 40 µL Calibrator/ Sample, incubate 2 hours at RT.
3. Wash with PBS-T. Add 25 µL Detection Antibodies.
4. Wash with PBS-T. Add 150 µL Read Buffer T, read.

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[14] Funicello, M., *et al.* ***Cathepsin K null mice show reduced adiposity during the rapid accumulation of fat stores.*** PLoS ONE. 2007 Aug 1;2(1):e683.


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O'Brien, P.J. ***Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity.*** Toxicology. 2008 245(3):206-18.


References using MSD MULTI-ARRAY technology

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| Assays | Description | Catalog Numbers |
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| *Clusterin | Rat Clusterin Kit (1 Plate)<br>Rat Clusterin Kit (5 Plates) | K153HXC-1<br>K153HXC-2 |
| *Skeletal Troponin I (sTnI) | Rat Skeletal Troponin I Kit (1 Plate)<br>Rat Skeletal Troponin I Kit (5 Plates) | K153IMC-1<br>K153IMC-2 |
| *TIMP-1 | Human TIMP-1 Kit (1 Plate)<br>Human TIMP-1 Kit (5 Plates) | K151JFC-1<br>K151JFC-2 |
| *Luteinizing Hormone (LH) | Human Luteinizing Hormone (LH) Kit (1 Plate)<br>Human Luteinizing Hormone (LH) Kit (5 Plates) | K151ETC-1<br>K151ETC-2 |
| *Argutus AKI Test®<br>αGST, GSTYb1, RPA-1 | Argutus AKI Test (rat) Kit (1 Plate)<br>Argutus AKI Test (rat) Kit (5 Plates) | K15156C-1<br>K15156C-2 |
| *Kidney Injury Panel 1<br>Albumin, TIM-1, Lipocalin-2, Osteopontin | Kidney Injury Panel 1 (rat) Kit (1 Plate)<br>Kidney Injury Panel 1 (rat) Kit (5 Plates) | K15162C-1<br>K15162C-2 |
| *Cardiac Injury Panel 2<br>cTnI, cTnT, FABP3 | Cardiac Injury Panel 2 (rat) Kit (1 Plate)<br>Cardiac Injury Panel 2 (rat) Kit (5 Plates) | K15155C-1<br>K15155C-2 |
| *Cardiac Injury Panel 3<br>cTnI, cTnT, FABP3, Myl3 | Cardiac Injury Panel 3 (rat) Kit (1 Plate)<br>Cardiac Injury Panel 3 (rat) Kit (5 Plates) | K15161C-1<br>K15161C-2 |
| *Muscle Injury Panel 1<br>cTnI, cTnT, FABP3, Myl3, sTnI | Muscle Injury Panel 1 (rat) Kit (1 Plate)<br>Muscle Injury Panel 1 (rat) Kit (5 Plates) | K15181C-1<br>K15181C-2 |
| *Acute Phase Protein Panel 1<br>A2M, AGP | Acute Phase Protein Panel 1 (rat) Kit (1 Plate)<br>Acute Phase Protein Panel 1 (rat) Kit (5 Plates) | K15175C-1<br>K15175C-2 |
| Steroid Hormone Panel 1<br>Estradiol, Progesterone, Testosterone, DHEA | Steroid Hormone Panel 1 (human, mouse, rat) Kit (1 Plate)<br>Steroid Hormone Panel 1 (human, mouse, rat) Kit (5 Plates) | K15178C-1<br>K15178C-2 |

** Qualified Kit*

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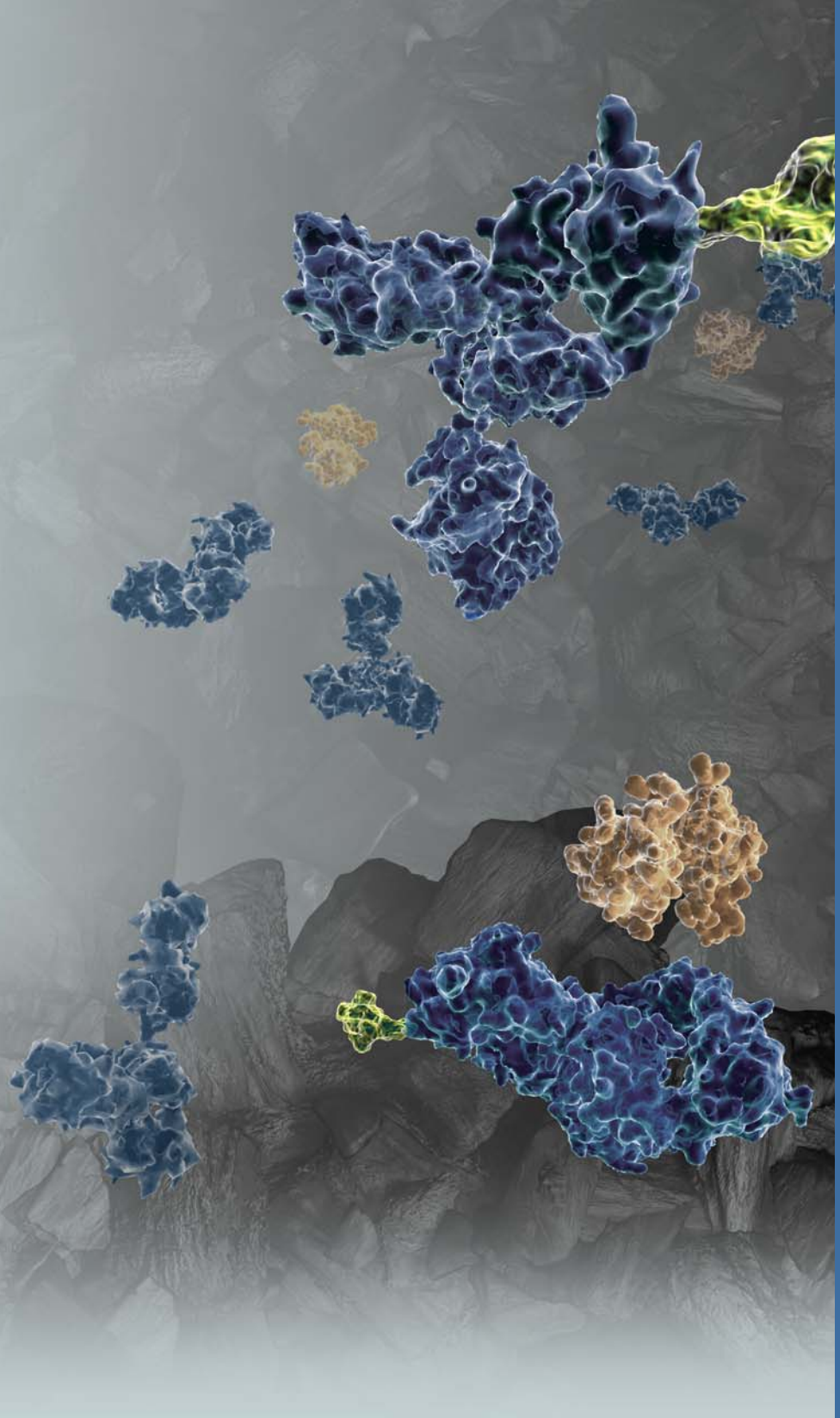

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