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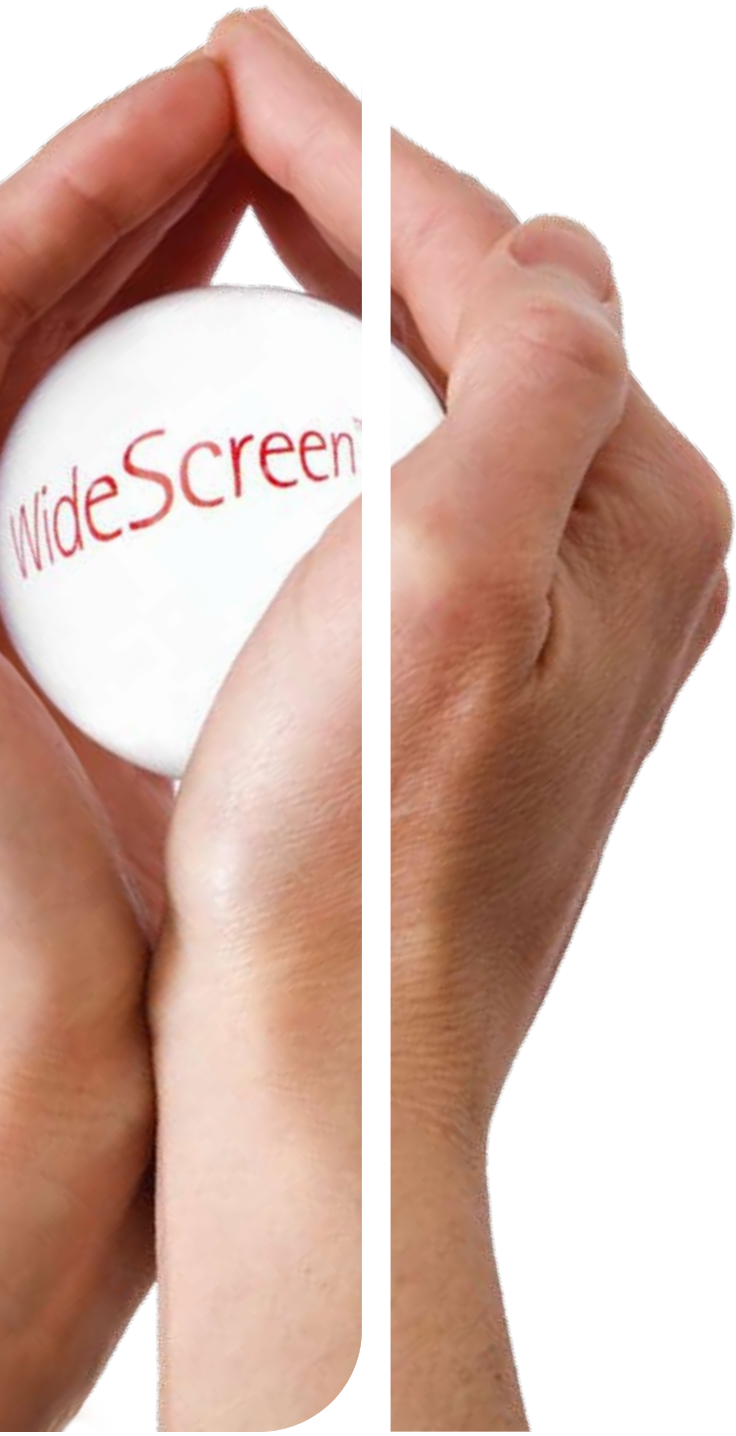


WideScreen™ Biomarker Assay Kits
co-developed with Rules Based Medicine

WideScreen
Rat Kidney Toxicity Assays

For the Luminex® xMAP® Technology platform

EMD

WideScreen™ Rat Kidney Toxicity Assays

The current approach to evaluating drug-induced nephrotoxicity in preclinical studies is to measure serum creatinine and blood urea nitrogen (BUN) levels in rats. The ability of the kidney to excrete creatinine and urea nitrogen decreases with damage, resulting in increased serum creatinine and BUN. The utility of creatinine and BUN as markers of kidney injury is currently under question. Serum levels of BUN are influenced by protein intake, and creatinine levels fluctuate as a function of age, muscle mass, and other clearance mechanisms. Moreover, serum creatinine and BUN levels rise only when significant kidney injury has occurred, compensatory regenerative mechanisms initially maintain kidney function during early stage damage. Creatinine and BUN level analysis is often combined with histopathological examination of kidney sections, a labor intensive terminal technique that hampers the ability to perform time course studies.

The Critical Path Institute, through the public-private Predictive Safety Testing Consortium (PSTC), initiated a program to develop improved testing methods to identify drug-induced renal damage. Rules Based Medicine (RBM) collaborated with the PSTC by developing assays and providing data on thousands of rat urine samples submitted for analysis by Novartis AG.

The results of the PSTC study were submitted to the FDA and EMEA in 2008, leading to the listing of seven urinary kidney damage biomarkers.

EMD, in partnership with RBM, has now released assay kits that include four of the new accepted biomarkers (KIM-1, β2-microglobulin (β2m), cystatin C, and clusterin), along with six other key protein markers of kidney injury (GST-α, TIMP-1, VEGF, calbindin, NGAL, and osteopontin).

Two assay panels are available:

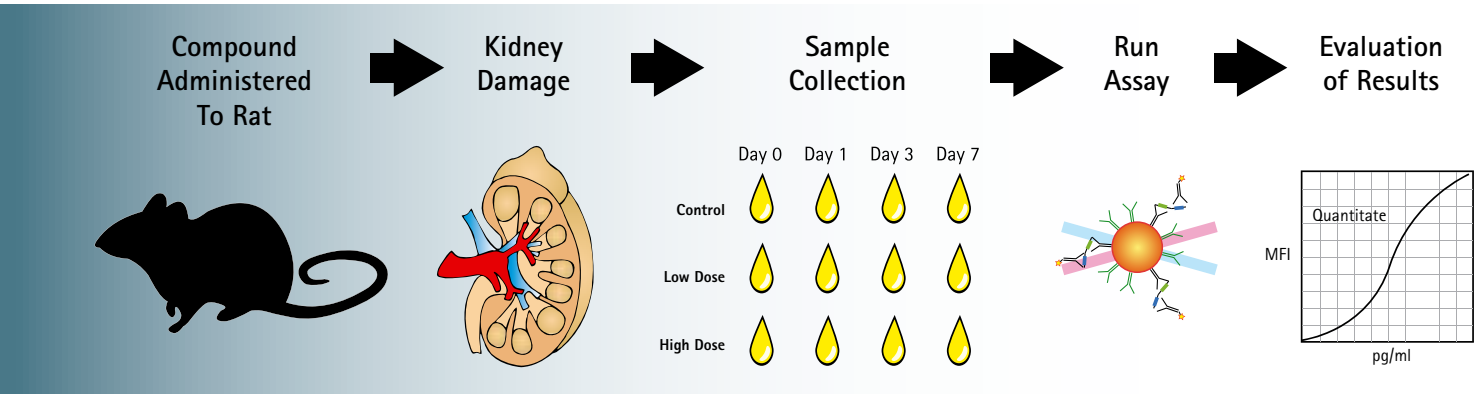
WideScreen™ Rat Kidney Toxicity Panel 1

- β2m
- GST-α
- KIM-1
- TIMP-1
- VEGF

WideScreen™ Rat Kidney Toxicity Panel 2

- Calbindin
- Clusterin
- Cystatin C
- NGAL
- Osteopontin

Experimental Workflow



WideScreen™ Rat Kidney Toxicity Panel 1

Protein	Function	Damaged region
β2m *β2-microglobulin*	Small cell surface protein shed into the blood and normally reabsorbed by the proximal tubules of the kidney. High β2m levels result from lack of efficient reabsorption due to renal failure.	Proximal tubule Glomerulus
GST-α *Glutathione S-transferase alpha*	Contributes to detoxification of a wide range of compounds including carcinogens, therapeutic drugs, and products of oxidative stress.	Proximal tubule
KIM-1 *Kidney injury molecule 1*	Membrane protein expressed at elevated levels after injury of proximal tubule epithelial cells due to ischemic renal damage.	Proximal tubule
TIMP-1 *Tissue inhibitor of matrix metalloproteinase-1*	Regulates extracellular matrix synthesis and degradation and, along with matrix metalloproteinases, is essential for tumor growth and health.	Proximal tubule Distal tubule
VEGF *Vascular endothelial growth factor*	Growth factor that induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis, and induces permeabilization of blood vessels. Upregulated in response to kidney injury.	

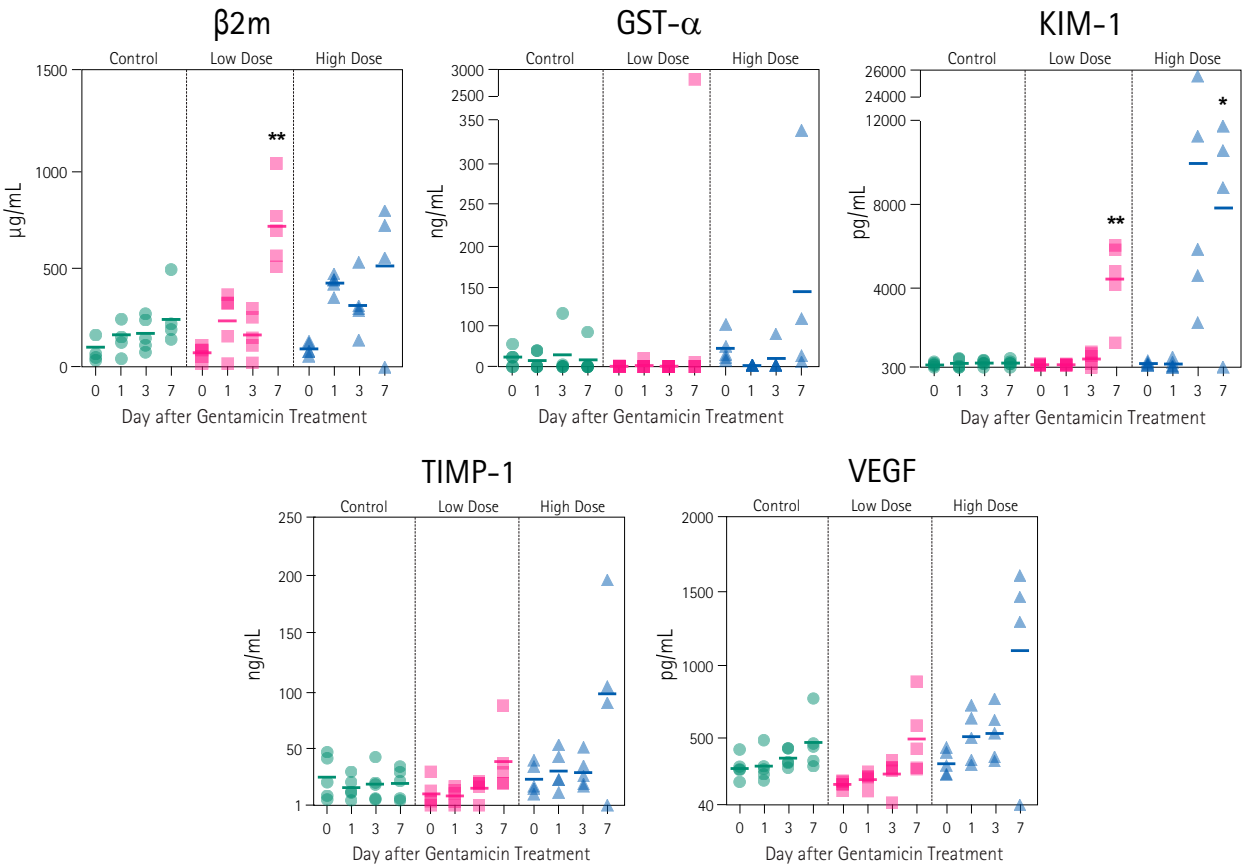
Performance Characteristics

Species	Rat, other species not tested
Sample Size	15 µl
Sample Types	Urine, plasma*
Intra-assay CV	0%-15%
Inter-assay CV	7%-15%
Cross-reactivity	Negligible (<1%)

*dilutions vary; see User Protocol TB522 for details.

Analyte	Standard Range	Assay LDD*	Average Recovery from Urine	Linearity of Dilution	
β2m	0.29-636 µg/ml	2.8 µg/ml	78%	1:4	90%
				1:8	85%
				1:16	97%
GST-α	1.9-4255 ng/ml	34 ng/ml	79%	1:4	89%
				1:8	82%
				1:16	95%
KIM-1	0.049-108 ng/ml	0.049 ng/ml	121%	1:4	108%
				1:8	116%
				1:16	104%
TIMP-1	0.011-24 ng/ml	0.011 ng/ml	73%	1:4	116%
				1:8	105%
				1:16	94%
VEGF	1.7-3788 pg/ml	1.7 pg/ml	106%	1:4	116%
				1:8	114%
				1:16	119%

*least detectable dose



Rat Kidney Toxicity Biomarker Levels in Urine Samples
The WideScreen™ Rat Kidney Toxicity Panels were used to investigate time- and dose-related changes in the concentration of nephrotoxicity biomarkers in urine from rats treated with the aminoglycoside antibiotic gentamicin for 7 days. Gentamicin was administered to rats (n = 5 per dose group) by s.c. injections twice daily at doses of 0 mg/kg bw (Control), 60 mg/kg bw (Low Dose), or 120 mg/kg bw (High Dose). Urine samples were collected using metabolic cages and stored at -80°C until analysis. All values were multiplied by the appropriate dilution factor to represent the undiluted sample

WideScreen™ Rat Kidney Toxicity Panel 2

Protein	Function	Damaged region
Calbindin	Calcium binding protein found in epithelial cells, including distal tubular cells and cortical collecting tubules of the kidney.	Proximal tubule Glomerulus
Clusterin *Apolipoprotein J*	Conserved protein induced during tissue injury or remodeling.	Proximal tubule Distal tubule
Cystatin C	Extracellular inhibitor of cysteine proteases normally expressed in vascular wall smooth muscle cells.	Glomerulus
NGAL *Neutrophil gelatinase associated lipocalin* *Lipocalin-2*	Expressed in kidney cells as protective mechanism during the inflammatory response.	Proximal tubule
Osteopontin	Multifunctional glycoprotein with key immunomodulatory roles such as enhancement IFN-γ and IL-12, and downregulation of IL-10 expression.	Proximal tubule Loop of Henle Distal tubule

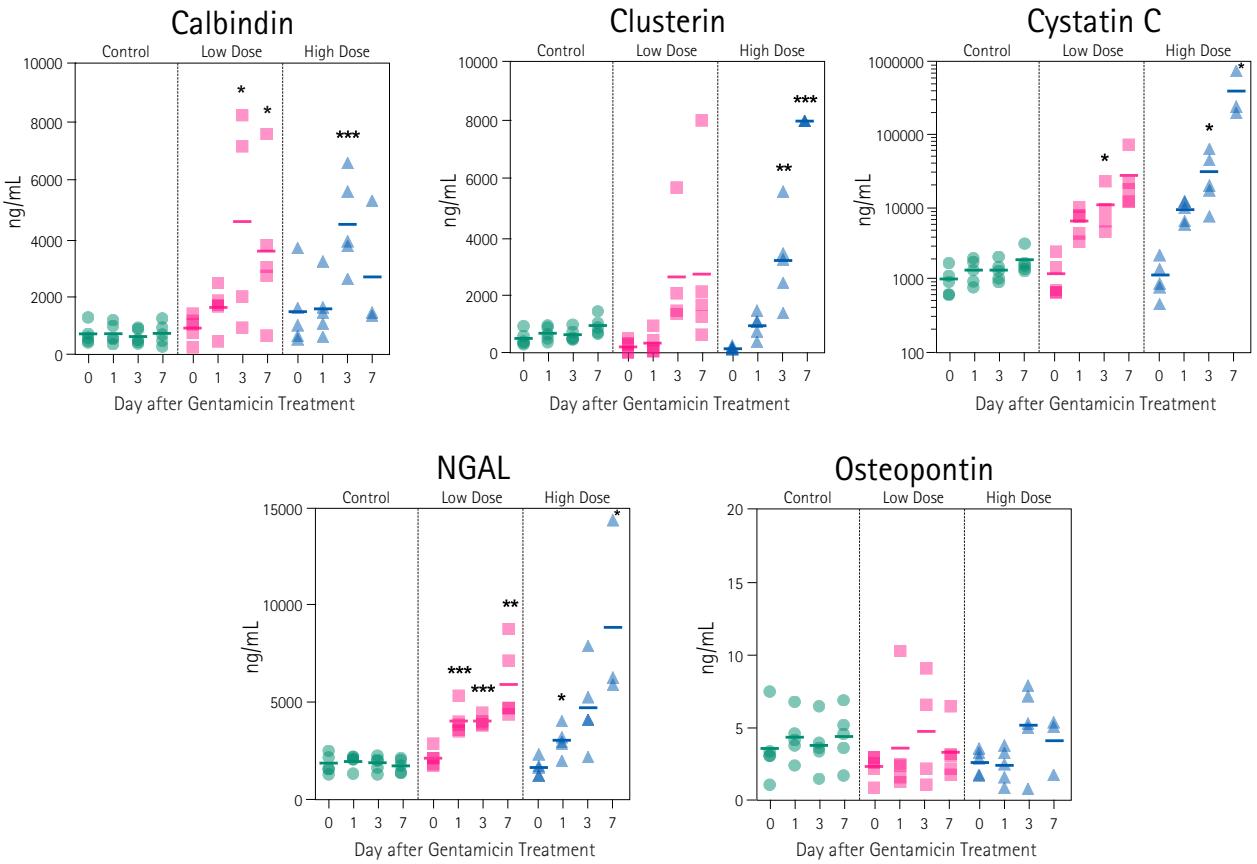
Performance Characteristics

Species	Rat, other species not tested
Sample Size	1 µl
Sample Types	Urine, plasma*
Intra-assay CV	0%-18%
Inter-assay CV	3%-16%
Cross-reactivity	Negligible (<1%)

*dilutions vary; see User Protocol TB523 for details.

Analyte	Standard Range	Assay LDD*	Average Recovery from Urine	Linearity of Dilution	
Calbindin	0.10-225 ng/ml	0.10 ng/ml	100%	1:100 1:200 1:400	99% 101% 108%
Clusterin	0.37-800 ng/ml	2.2 ng/ml	92%	1:100 1:200 1:400	106% 131% 106%
Cystatin C	0.021-45 ng/ml	0.021 ng/ml	101%	1:100 1:200 1:400	98% 97% 95%
NGAL	0.91-2000 ng/ml	4.9 ng/ml	115%	1:100 1:200 1:400	101% 118% 105%
Osteopontin	0.014-30 ng/ml	0.014 ng/ml	107%	1:100 1:200 1:400	115% 115% 124%

*least detectable dose

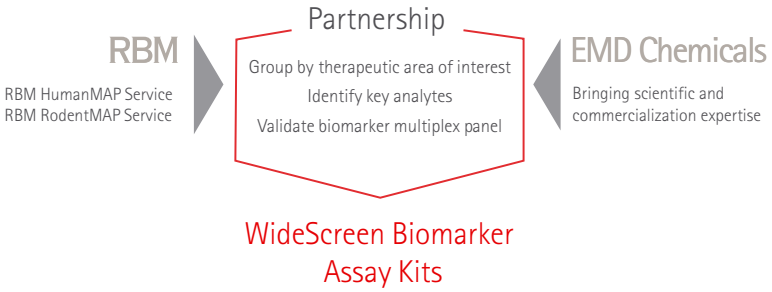


concentration. Data points at or below the corrected least detectable dose (LDD) were plotted on the x-axis. Statistically significant changes compared to controls are denoted as: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Kidney toxicity markers β2m and KIM-1 were found at significantly elevated levels in the gentamicin-treated animals (day 7), indicating renal damage. Many animals also showed elevated levels of GST-α, TIMP-1 and VEGF by day 7, but these differences fall short of statistical significance due to the variability of values within the small sample size. Data were generated in collaboration with D. Hoffmann and A. Mally (Department of Toxicology, University of Würzburg, Germany).

WideScreen Rat Kidney Toxicity Assays

In 2008 the Predictive Safety Testing Consortium (PSTC), a public-private consortium led by the Critical Path Institute (C-Path) submitted a list of urinary biomarkers indicative of drug-induced kidney damage to the FDA and EMEA regulatory authorities. The FDA and EMEA have issued new guidelines on the submission of the biomarkers as indicators of kidney damage in pre-clinical studies.

Rules Based Medicine worked with the members of the PSTC to develop the assays used in the kidney toxicity study, and made the assays available in the Rat Kidney MAP testing service. EMD and Rules Based Medicine have collaborated to develop these assays as commercially available kits, exclusively for the Luminex® xMAP® Technology platform, to support preclinical rat nephrotoxicity studies



WideScreen™ Assays using xMAP® Technology are immunosandwich assays immobilized on microparticle beads that are detected using a Luminex instrument (e.g. Luminex 100 IS™ or 200™ Systems). Using uniquely identifiable beads, multiple protein targets can be simultaneously quantified from a single sample. The Luminex instrument employs advanced fluidics and dual lasers to detect the bead identity and the amount of bound reporter. Standard curves generated using purified proteins enable the quantification of experimental samples.

WideScreen™ Rat Kidney Toxicity Panel 1

Cat. No. 72164-3 **96 Tests**

A pre-mixed multiplex bead kit of quantitative antibody-based assays for simultaneous detection of five biomarkers of kidney damage in rat: β2m, GST-α, KIM-1, TIMP-1, and VEGF.

The kit includes all the reagents and buffers needed to analyze the above proteins in urine using the Luminex® xMAP® System:

- Rat Kidney Toxicity Panel 1 Capture Beads
- Rat Kidney Toxicity Panel 1 Detection Antibodies
- Rat Kidney Toxicity Panel 1 Standards Mix
- Rat Kidney Toxicity Panel 1 Control 1
- Rat Kidney Toxicity Panel 1 Control 2
- 1X Assay Buffer Type 2
- Rat Kidney Toxicity Panel 1 Blocking Buffer
- 1X Sample Dilution Buffer Type 3
- Standard Curve Diluent Type 4
- 15X Streptavidin-Phycoerythrin
- 96-well Filter Plate and Sealer

WideScreen™ Rat Kidney Toxicity Panel 2

Cat. No. 72174-3 **96 Tests**

A pre-mixed multiplex bead kit of quantitative antibody-based assays for simultaneous detection of five biomarkers of kidney damage in rat: calbindin, clusterin, cystatin C, NGAL, and osteopontin.

The kit includes all the reagents and buffers needed to analyze the above proteins in urine using the Luminex® xMAP® System:

- Rat Kidney Toxicity Panel 2 Capture Beads
- Rat Kidney Toxicity Panel 2 Detection Antibodies
- Rat Kidney Toxicity Panel 2 Standards Mix
- Rat Kidney Toxicity Panel 2 Control 1
- Rat Kidney Toxicity Panel 2 Control 2
- 1X Assay Buffer Type 2
- Blocking Buffer Type 4
- 1X Sample Dilution Buffer Type 3
- Standard Curve Diluent Type 5
- 15X Streptavidin-Phycoerythrin
- 96-well Filter Plate and Sealer

Due to different sample dilution requirements WideScreen™ Rat Kidney Toxicity Panels 1 and 2 should not be multiplexed together

THE POWER INSIDE Luminex — xMAP TECHNOLOGY Because you want to know more

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