

Acute Phase Response
Cancer
Cardiovascular Disease
Cytokines, Chemokines,
Growth Factors
Diabetes
Toxicology
Genotyping
Immunoglobulin Isotyping
Signal Transduction

Bio-Plex Pro™ RBM Multispecies Kidney Toxicity Panels

MAGNETIC SEPARATION ENABLED

Albumin, B2M, Calbindin, Clusterin, Cystatin C, GST-π, IL-18, KIM-1, MCP-1, NGAL, Osteopontin, TFF3

- Assays available for human, canine, and rat models
- Magnetic workflow
- All-in-one kit format
- 2-level quality controls



High-Performance Multiplex Immunoassays for Kidney Toxicology Research

The Bio-Plex Pro RBM kidney toxicity assays, developed in partnership with Myriad RBM, comprise a highly relevant set of biomarkers for early detection and characterization of kidney toxicity/injury. Myriad RBM's close collaboration with the Predictive Safety Testing Consortium (PSTC), FDA, and EMEA was instrumental in the selection of markers found in these panels.

The assays are built on magnetic beads to enable robust quantification of multiple proteins in human, canine, and rat urine samples, providing valuable information throughout drug development — from lead optimization to preclinical and clinical protocol decision making. Assays are offered as premixed all-in-one kits for research involving:

- Drug-induced kidney toxicity
- Characterization of kidney injury associated with medical conditions:
 - Diabetes
 - High blood pressure
 - Glomerulonephritis
 - Polycystic kidney disease
 - Chronic infection
 - SLE (lupus) and other autoimmune disorders

Assay Features

- Optimized for high precision and lot-to-lot reproducibility of sample measurements
- Magnetic beads for simplified plate processing
- 2-level quality controls with kit lot-specific ranges
- Assay quick guide to get you started right away
- Compatible with Bio-Plex® 200, Bio-Plex 3D, and Bio-Plex® MAGPIX™ systems

Assay Performance Definitions

The following parameters are indicative of assay performance as shown in Table 2.

Assay working range — the range of concentrations within which the assay is precise and accurate. Boundaries of the assay working range are defined by the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ)

Precision — the coefficient of variation (%CV) at concentrations within the assay working range

Accuracy (recovery) — percentage of the observed concentration relative to the expected concentration of a known amount of analyte within the assay working range

Sensitivity (limit of detection, LOD) — the concentration of analyte for which the fluorescence intensity signal is two standard deviations above the background signal

BIO-RAD

Table 1. Bio-Plex Pro RBM kidney toxicity analytes.

Analyte	Analyte Description	Damaged Kidney Region
Albumin	Approximately 99% of filtered albumin is reabsorbed in the proximal tubule of kidney nephrons. In pathological conditions when glomerular capillary wall permeability and/or filtration rate increases, albumin excretion in urine increases. Detection of increased urinary albumin is of particular importance in the study of incipient renal disease. Higher levels of urinary albumin are also associated with cardiovascular disease in individuals with diabetes or hypertension.	Proximal tubule
B2M	Beta-2-microglobulin is a protein found on the surface of all nucleated cells and shed into the blood. Due to its small size, it passes through the glomerular membrane, but normally less than 1% is excreted due to reabsorption in the proximal tubules of the kidney. Elevated urine levels are an early biomarker of acute drug-induced glomerular damage resulting in impairment of kidney tubular reabsorption.	Glomerulus
Calbindin	Calbindins are calcium-binding proteins belonging to the troponin C superfamily. Calbindin D-28K is found predominantly in subpopulations of central and peripheral nervous system neurons, in epithelial cells involved in Ca ²⁺ transport, such as distal tubular cells and cortical collecting tubules of the kidney, and in enteric neuroendocrine cells.	Distal tubule
Clusterin	Clusterin (also known as ApoJ) is a highly conserved protein expressed in apoptotic heart, brain, lung, liver, kidney, and other tissues. Urinary clusterin is an early biomarker of acute drug-induced tubular damage and is more sensitive and specific than serum creatinine (Scr) or blood urea nitrogen (BUN) in monitoring renal function after exposure to nephrotoxicants.	Proximal tubule, distal tubule
Cystatin C	Cystatin C is a 13 kD protease inhibitor with high levels of expression in testis, epididymis, prostate, seminal vesicle, and vascular wall smooth muscle cells. Urine cystatin C is an early biomarker of acute drug-induced glomerular alterations or damage resulting from impairment of kidney tubular reabsorption.	Glomerulus
GST-π	The glutathione S-transferase (GST) superfamily comprises cytosolic dimeric isoenzymes of 45–55 kD in size. GST-π is located in the distal tubule and urinary levels are increased with distal tubule damage and can be indicative of renal transplant rejection, kidney infection, diabetes, and chronic renal injury.	Distal tubule
IL-18	Interleukin-18 is a proinflammatory cytokine that induces interferon-gamma production in T cells and natural killer cells. Elevated urinary levels are an early marker of acute kidney injury (AKI), chronic renal insufficiency, endotoxemia, cancer chemotherapeutic toxicity, allograft rejection, and ischemia-reperfusion injury, and are an independent predictor of mortality in critically ill patients.	Proximal and distal tubule
KIM-1	Kidney injury molecule-1 (also known as TIM-1) is an immunoglobulin superfamily cell surface protein that is highly upregulated on the surface of injured kidney epithelial cells and is localized predominantly to the apical membrane of the surviving proximal epithelial cells.	Proximal tubule
MCP-1	Monocyte chemoattractant protein-1 plays a role in the recruitment of monocytes to sites of injury and infection. MPC-1 is elevated in the urine of people with lupus and is a warning sign of inflammation of the kidney. There is a close association between albuminuria, urinary MCP-1, and interstitial macrophage infiltration with progression of renal injury before the establishment of advanced renal scarring.	Proximal tubule
NGAL	Neutrophil gelatinase-associated lipocalin (also known as lipocalin-2) is a small protein found not only in neutrophils but also in certain epithelia, such as renal tubules, where its expression is dramatically increased in ischemic or nephrotoxic injury. NGAL levels rise in urine and blood within 2 hours of renal insult, making NGAL a biomarker for acute renal injury.	Proximal tubule
Osteopontin	Osteopontin (OPN) is a cytokine involved in the Th1 immune response that is secreted by a variety of tissues upon injury. It is present in human urine at levels that can effectively inhibit calcium oxalate crystallization. Low concentrations of OPN have been documented in urine from patients with renal stone disease compared to normal individuals.	Proximal tubule, loop of Henle, distal tubule, collecting duct
TFF3	Trefoil factor 3 exists as monomeric (6.7 kD) and dimeric (13.1 kD) forms expressed by goblet cells of the intestine and the colon, and in the human respiratory tract, promoting survival and differentiation of epithelial cells. TFF3 may show reduced urine excretion in response to acute kidney injury. Higher urinary levels may indicate ongoing kidney repair and increased risk for chronic kidney disease (CKD).	Proximal tubule

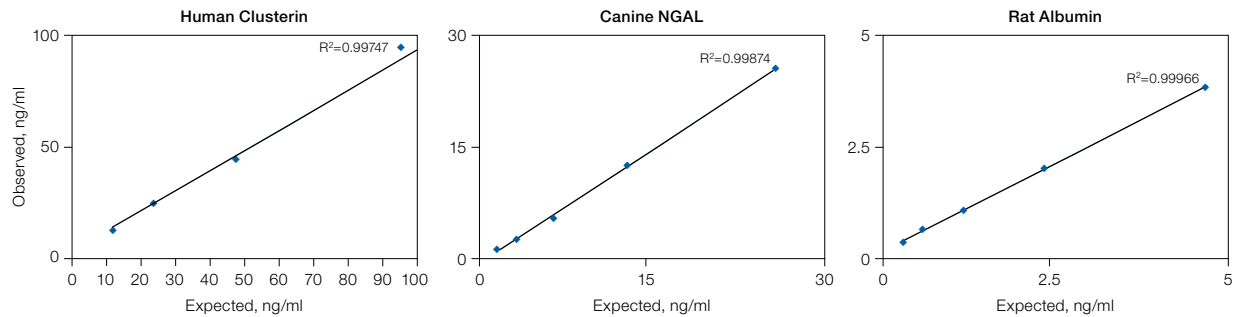


Fig. 1. Linearity of dilution was examined by spiking recombinant analyte into urine samples collected from human, canine, and rat respectively. The spiked samples were serially diluted to obtain at least four data points. The observed and expected analyte concentrations were plotted and the correlation coefficient (R^2) values reflect linearity in signal response.

Table 2. Representative assay performance.

Assay Panel	Analyte	Alternate Names	Bead Region	Assay Working Ranges, ng/ml		Assay Sensitivity, ng/ml	Assay Precision	
				LLOQ	ULOQ	LOD	Intra-assay %CV	Inter-assay %CV
Canine Kidney Toxicity Panel 1	Clusterin	ApoJ	12	0.69	800	0.65	4	6
	KIM-1	TIM-1	53	0.012	10	0.0046	4	7
	MCP-1		15	0.013	4.8	0.0043	5	12
	NGAL	Lipocalin-2	46	0.010	9.0	0.0042	5	7
Canine Kidney Toxicity Albumin Kit	Albumin		30	0.0052	4.8	0.0022	8	11
Human Kidney Toxicity Panel 1	Calbindin		64	4.4	1,750	0.97	4	5
	Clusterin	ApoJ	12	1.3	1,250	0.57	3	10
	GST- π		25	0.5	230	0.23	5	8
	IL-18		21	0.019	15	0.048	5	7
	KIM-1	TIM-1	44	0.021	21	0.01	4	5
	MCP-1		15	0.011	3.8	0.0017	3	8
Human Kidney Toxicity Panel 2	Albumin		30	2.8	640	1.2	2	8
	B2M		22	0.043	22	0.022	3	9
	Cystatin C		51	0.16	40	0.077	3	20
	NGAL	Lipocalin-2	46	0.062	34	0.052	3	8
	Osteopontin	OPN	20	3.8	2,100	1.7	6	12
	TFF3		61	0.075	98	0.036	2	6
Rat Kidney Toxicity Panel 1	Clusterin	ApoJ	12	0.99	840	0.43	3	12
	IL-18		21	0.36	35	0.17	4	5
	KIM-1	TIM-1	20	0.042	50	0.023	4	7
	MCP-1		15	0.15	35	0.045	2	6
	Osteopontin	OPN	52	0.0039	3.2	0.0015	4	5
Rat Kidney Toxicity Panel 2	B2M		22	8.3	2,760	4.1	10	18
	Calbindin		62	0.7	535	0.61	9	14
	Cystatin C		44	0.062	42	0.026	2	6
	NGAL	Lipocalin-2	46	0.63	480	0.22	5	10
Rat Kidney Toxicity Albumin Kit	Albumin		30	0.043	35	0.014	5	9

The LLOQ, ULOQ, LOD, and inter-assay precision %CV are mean data determined from three independent multiplex assays. Intra-assay %CV is the mean of eight standard points run in triplicate within one representative assay. LLOQ and ULOQ are defined as the boundary standard curve points that meet precision and accuracy specifications of $\leq 30\%$ intra-assay CV and 80–120% recovery. Data were generated using the magnetic workflow with the Bio-Plex Pro II wash station.

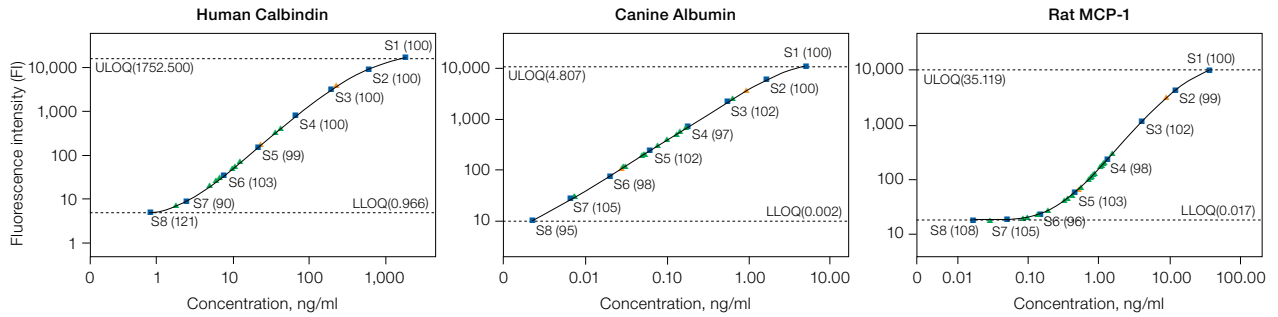


Fig. 2. Standard curves with assay controls and urine samples. Standard points were prepared by serially diluting a reconstituted standard threefold to generate an eight-point standard curve. Standard points with % recovery (■); controls (▲); samples (▲). Data were generated in Bio-Plex Manager™ software.

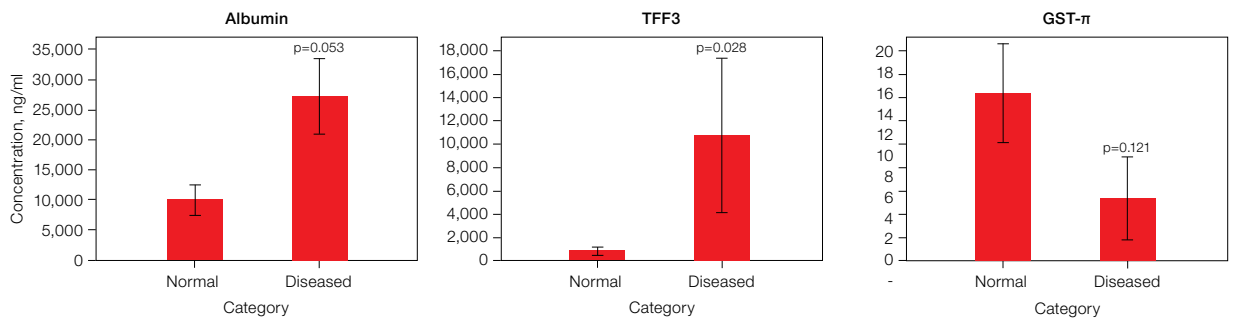


Fig. 3. Human samples — urine collected from healthy donors and donors with one or more of the following conditions: chronic kidney disease, diabetic nephropathy, kidney stones, and renal failure.

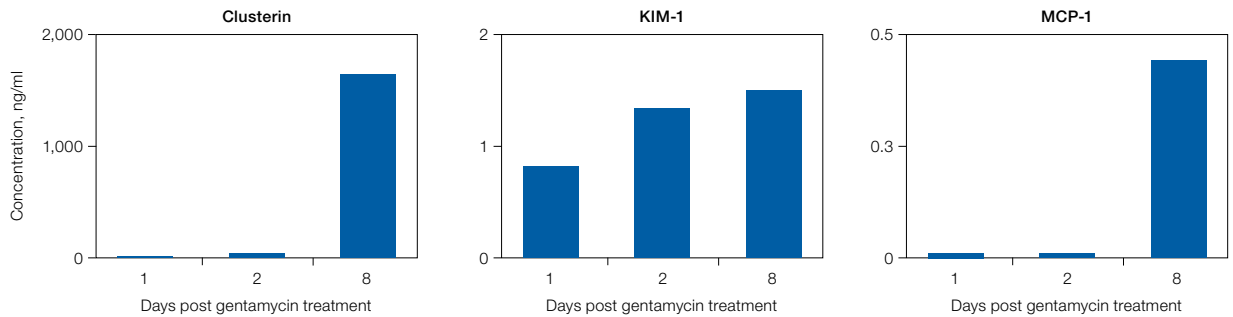


Fig. 4. Canine samples — urine collected from a canine 1, 2, and 8 days post gentamycin treatment.

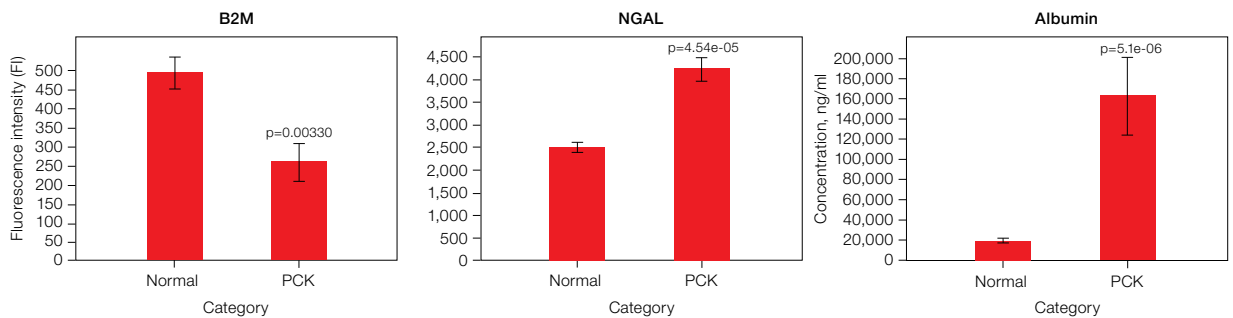


Fig. 5. Rat samples — urine collected from healthy Sprague Dawley rats and rats with polycystic kidney disease.

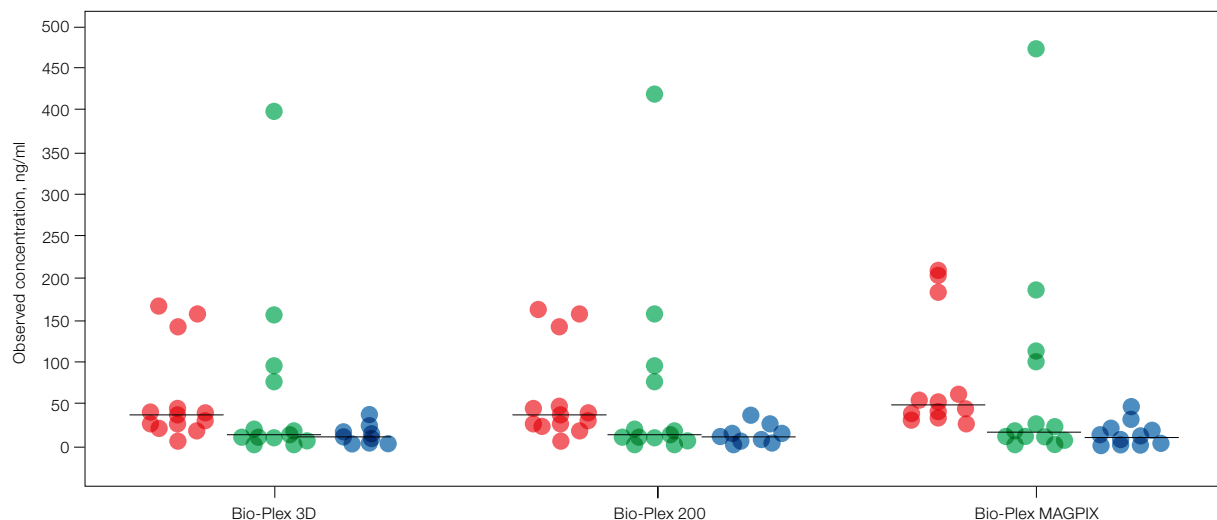


Fig. 6. Alignment of values from human urine samples across all Bio-Plex platforms. Calbindin (●); clusterin (●); GST-π (●). Data were generated in Bio-Plex Data Pro™ software.

Ordering Information

Catalog # Description

Bio-Plex Pro RBM Kidney Toxicity Premixed All-In-One Kits,

include premixed magnetic capture beads, premixed detection antibodies, standards mix, 2-level controls, blocking buffer, standard diluent, sample dilution buffer, 10x assay buffer, 10x streptavidin-PE, 96-well flat bottom plate, plate seals, and instructions

171-ATR1CK	Bio-Plex Pro RBM Human Kidney Toxicity Panel 1, 1 × 96-well, for the detection of the following analytes: calbindin, clusterin, GST-π, IL-18, KIM-1, MCP-1
171-ATR2CK	Bio-Plex Pro RBM Human Kidney Toxicity Panel 2, 1 × 96-well, for the detection of the following analytes: albumin, B2M, cystatin C, NGAL, osteopontin, TFF3
171-QTR1CK	Bio-Plex Pro RBM Canine Kidney Toxicity Panel 1, 1 × 96-well, for the detection of the following analytes: clusterin, KIM-1, MCP-1, NGAL
171-QTR2CK	Bio-Plex Pro RBM Canine Kidney Toxicity Albumin Kit, 1 × 96-well
171-KTR1CK	Bio-Plex Pro RBM Rat Kidney Toxicity Panel 1, 1 × 96-well, for the detection of the following analytes: clusterin, IL-18, KIM-1, MCP-1, osteopontin
171-KTR2CK	Bio-Plex Pro RBM Rat Kidney Toxicity Panel 2, 1 × 96-well, for the detection of the following analytes: B2M, calbindin, cystatin C, NGAL
171-KTR3CK	Bio-Plex Pro RBM Rat Kidney Toxicity Albumin Kit, 1 × 96-well

Wash Stations and Accessories

300-34376	Bio-Plex Pro Wash Station, microplate wash station for magnetic bead-based assays, includes magnetic plate carrier, waste bottle, 2 liquid bottles
300-34377	Bio-Plex Pro II Wash Station, microplate wash station for magnetic and polystyrene bead-based assays, includes magnetic plate carrier, vacuum manifold plate carrier, waste bottle, 2 buffer bottles
171-020100	Bio-Plex Handheld Magnetic Washer, includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays
171-025001	Bio-Plex Pro Flat Bottom Plates, pkg of 40, 96-well plates, for use with Bio-Plex Pro wash stations when using magnetic bead-based assays

Software

171-001510	Bio-Plex Data Pro Software with Bio-Plex Manager Software, Bio-Plex Data Pro software (5 seats), for multi-experiment analysis and advanced data visualization, and Bio-Plex Manager software (5 seats), for instrument data evaluation and optimization. CDs and security HASP key included
171-001513	Bio-Plex Data Pro Software, (5 seats), for multi-experiment analysis and advanced data visualization
171-STND01	Bio-Plex Manager Software, includes 1 user desktop license, for analysis of Bio-Plex data and generation of protocols, does not operate the instrument

The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

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