

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

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

High-Performance Multiplex Immunoassays for Kidney Toxicology Research

Bio-Plex Pro RBM Kidney Toxicity Assays, developed in partnership with Myriad RBM, are composed of a highly relevant set of biomarkers for early detection and characterization of kidney toxicity/injury (Table 1). The close collaboration of Myriad RBM with the Predictive Safety Testing Consortium, U.S. Food and Drug Administration, and agencies in Europe, the Middle East, and Africa 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 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
 - Systemic lupus erythematosus (SLE) 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 kit lot-specific quality controls
- Assay quick guide to get you started right away
- Compatible with Bio-Plex 200, Bio-Plex 3D, and Luminex MAGPIX and INTELLIFLEX Systems

Assay Performance Data and Definitions

These parameters are indicative of assay performance (Table 2). Assay performance data are shown in Figures 1–5.

- **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 2 standard deviations above the background signal

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 tubules of kidney nephrons. In pathological conditions in which 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 levels in urine are an early biomarker of acute drug-induced glomerular damage, which results 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 and 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. Urinary 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 is composed of cytosolic dimeric isoenzymes of 45–55 kD in size. GST-π is located in the distal tubule and its levels in urine are increased with distal tubule damage. This 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 levels in urine 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 tubule 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. MCP-1 is elevated in the urine of people with SLE and is a warning sign of inflammation of the kidney. Albuminuria, urinary MCP-1, and interstitial macrophage infiltration are closely associated 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. Elevated levels of urine may indicate ongoing kidney repair and increased risk for chronic kidney disease (CKD).	Proximal tubule

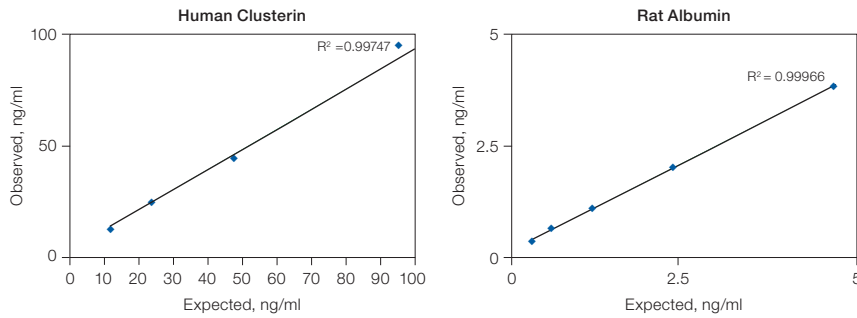


Fig. 1. Linearity of dilution was examined by spiking recombinant analyte into urine samples collected from human and rat. The spiked samples were serially diluted to obtain at least four data points. The observed and expected analyte concentrations were plotted and the coefficient of determination (R^2) 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
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 Wash Station. CV, coefficient of variation; LLOQ, lower limit of quantification; LOD, limit of detection; ULOQ, upper limit of quantification.

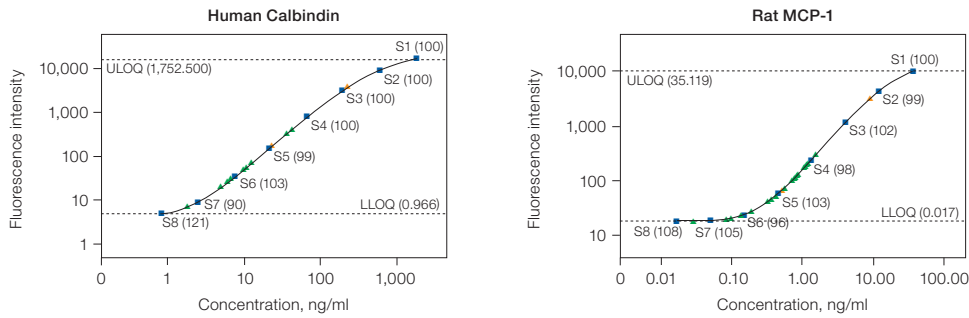


Fig. 2. Standard curves with assay controls and urine samples. Standard points were prepared by serially diluting a reconstituted standard threefold to generate an eight-point standard curve. Standard points with % recovery (■); controls (▲); samples (▲). Data were generated in Bio-Plex Manager Software. LLOQ, lower limit of quantification; S, standard; ULOQ, upper limit of quantification.

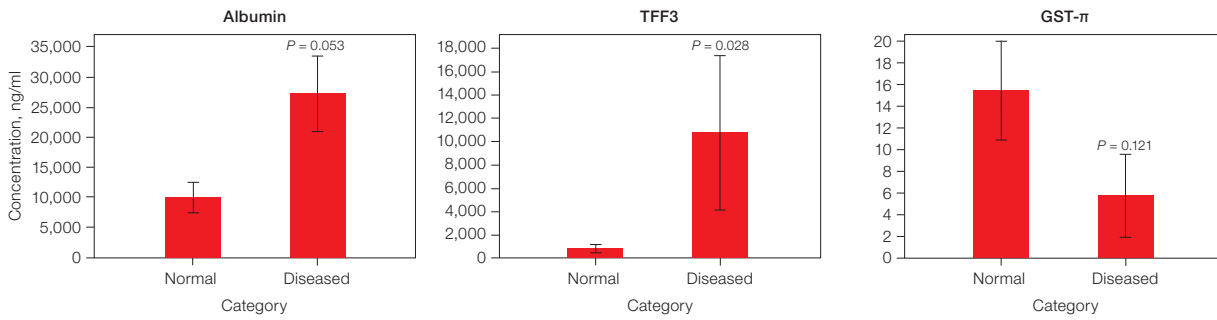


Fig. 3. Human samples. Urine collected from healthy donors and donors with one or more of the following conditions: chronic kidney disease, diabetic nephropathy, kidney stones, and renal failure.

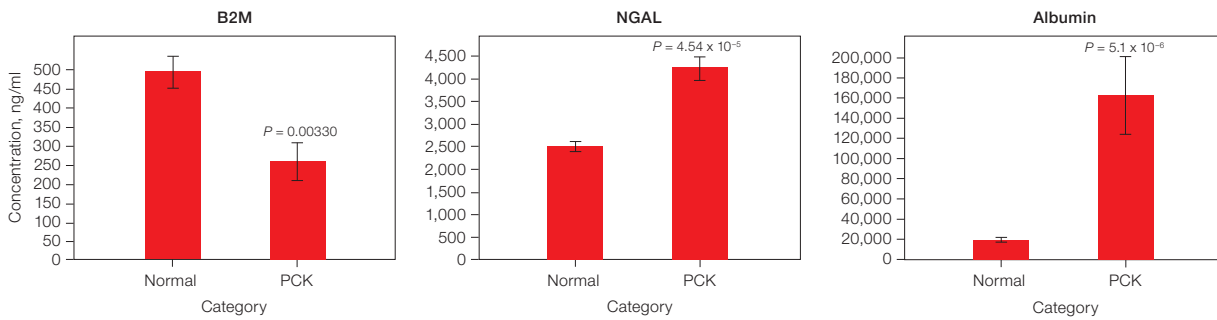


Fig. 4. Rat samples. Urine collected from healthy Sprague Dawley rats and rats with polycystic kidney disease (PCK).

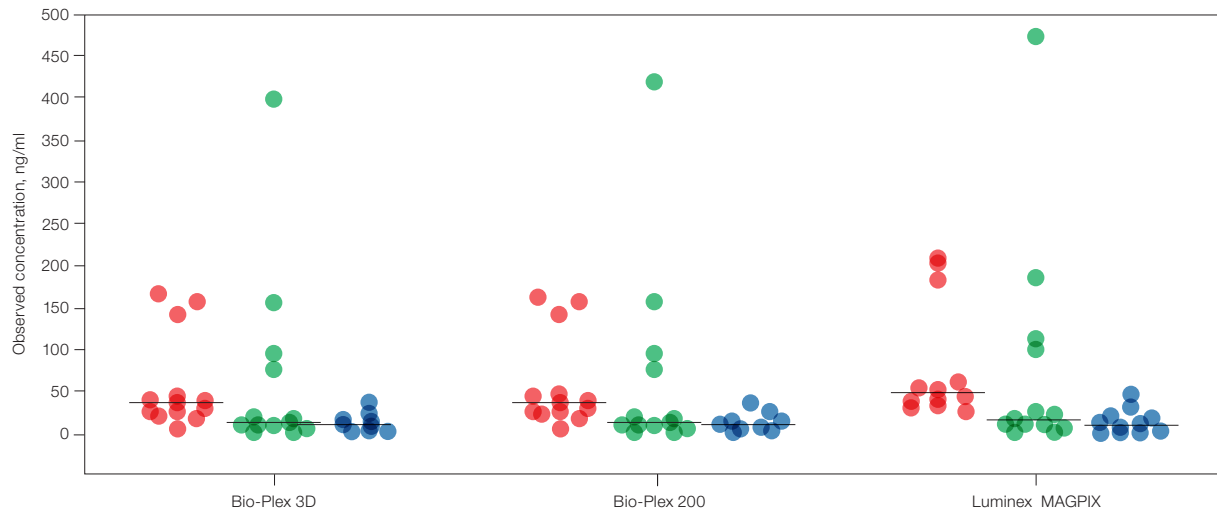


Fig. 5. Alignment of values from human urine samples across all Bio-Plex platforms. Calbindin (●); clusterin (●); GST-π (●). Data were generated in Bio-Plex Data Pro Software.

Ordering Information

Catalog #	Description
Bio-Plex Pro RBM Kidney Toxicity Premixed All-in-One Kits	
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-phycoerythrin, 96-well flat bottom plate, plate seals, and instructions.	
171ATR1CK	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
171ATR2CK	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
171KTR1CK	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
171KTR2CK	Bio-Plex Pro RBM Rat Kidney Toxicity Panel 2 , 1 × 96-well, for the detection of the following analytes: B2M, calbindin, cystatin C, NGAL
171KTR3CK	Bio-Plex Pro RBM Rat Kidney Toxicity Albumin Kit , 1 × 96-well
Wash Station and Accessories	
30034376	Bio-Plex Pro Wash Station , microplate wash station for magnetic bead-based assays, includes magnetic plate carrier, waste bottle, 2 buffer bottles
171020100	Bio-Plex Handheld Magnetic Washer , includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex Magnetic Assays
171025001	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

Catalog #	Description
Software	
12015390	Bio-Plex Data Pro Software , for multi-experiment analysis and advanced data visualization, sharing, and analysis
171STND01	Bio-Plex Manager Software , includes 1 user desktop license, for analysis of Bio-Plex data and generation of protocols, does not operate the instrument

Visit bio-rad.com/Bio-PlexKidneyTox for more information.

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