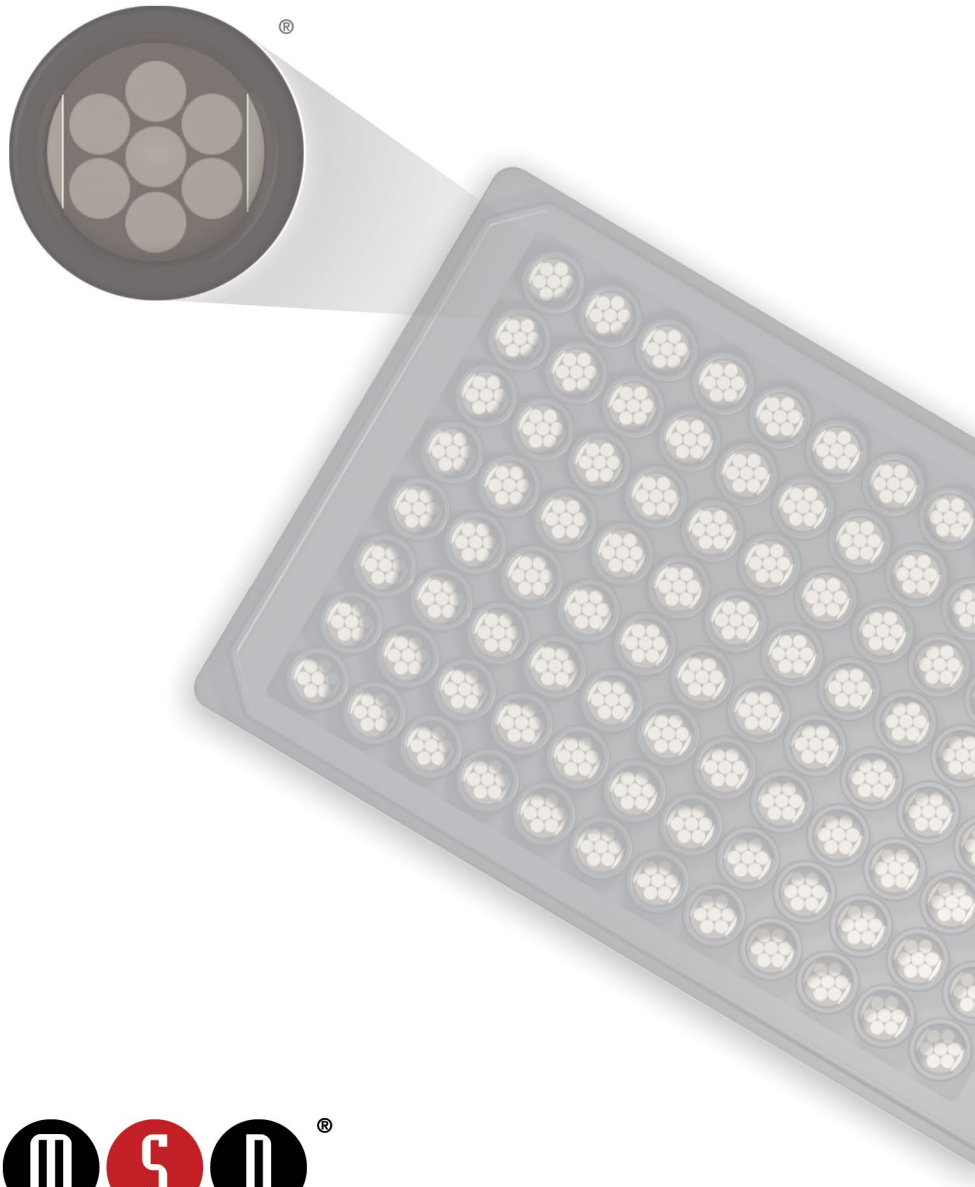
MSD® MULTI-SPOT Assay System

Kidney Injury Panel 3 (human) Kit

Calbindin, Clusterin, HAVCR1/KIM-1, Osteoactivin, TFF3, VEGF-A

T-PLEX®



	Catalog No
Multiplex Kit	K15189D

MSD®

www.mesoscale.com®

MSD Toxicology Assays

Kidney Injury Panel 3 (human) Kit

Calbindin, Clusterin, HAVCR1/KIM-1, Osteoactivin, TFF3, VEGF-A

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®

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Introduction

Measurement of protein biomarkers as indicators of drug-induced kidney toxicity shows promise for improving drug safety and accelerating development timelines. MSD produces high-performance multiplex panels to measure biomarkers of kidney injury. Multiple exploratory biomarkers of kidney toxicity are measured to determine their relative abundance in urine and their correlation with the severity and location of renal damage. MSD offers the Kidney Injury Panel 3 (human) Kit for monitoring levels of **Calbindin**, **Clusterin**, **HAVCR1/KIM-1**, **Osteoactivin**, **TFF3**, and **VEGF-A** (formerly named VEGF) in human samples.

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Kidney Injury Panel 3 (human) Kit are sandwich immunoassays (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD™ SULFO-TAG) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD® buffer that creates the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD® instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample.

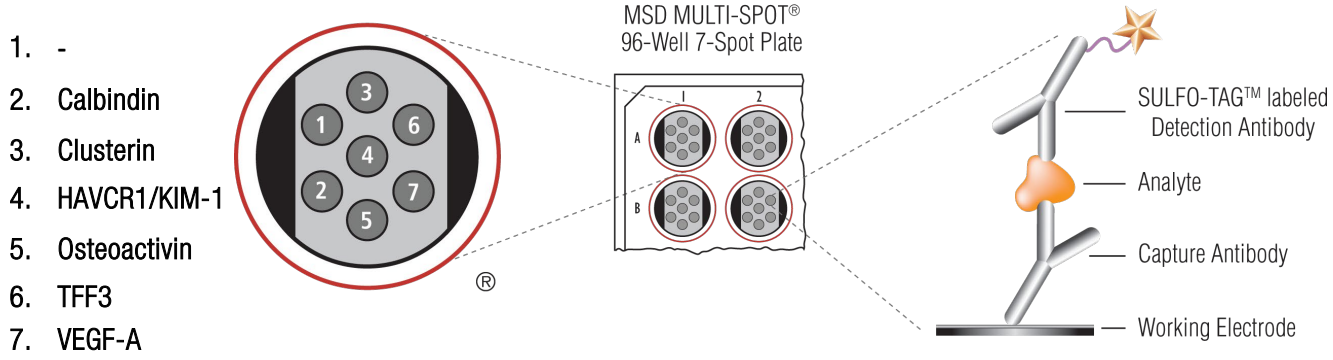

Figure 1. *Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.*

Reagents Supplied

Table 1. *Reagents that are supplied with the Kidney Injury Panel 3 (human) Kit*

Reagent	Storage	Catalog No.	Size	Quantity Supplied		
				1 Plate	5 Plates	25 Plates
MULTI-SPOT® 96-Well 7-Spot Kidney Injury Panel 3 (human) Plate‡	2–8 °C	N75189A-1	1 vial	1 vial	5 vials	25 vials
Anti-human Calbindin Antibody (50X)	2–8 °C	D21KS-2	75 µL	1 vial	—	—
		D21KS-3	375 µL	—	1 vial	5 vials
Anti-human Clusterin Antibody (50X)	2–8 °C	D21HX-2	75 µL	1 vial	—	—
		D21HX-3	375 µL	—	1 vial	5 vials
Anti-human HAVCR1/KIM-1 Antibody (50X)	2–8 °C	D21JH-2	75 µL	1 vial	—	—
		D21JH-3	375 µL	—	1 vial	5 vials
Anti-human Osteoactivin Antibody (50X)	2–8 °C	D21KT-2	75 µL	1 vial	—	—
		D21KT-3	375 µL	—	1 vial	5 vials
Anti-human TFF3 Antibody (50X)	2–8 °C	D21KU-2	75 µL	1 vial	—	—
		D21KU-3	375 µL	—	1 vial	5 vials
Anti-human VEGF-A Antibody (50X)‡	2–8 °C	D21KL-2	75 µL	1 vial	—	—
		D21KL-3	375 µL	—	1 vial	5 vials
Kidney Injury Panel 3 (human) Calibrator Blend (20X)	≤–70 °C	C0189-2	20 µL	1 vial	5 vials	25 vials
Diluent 37	≤–10 °C	R50AF-3	25 mL	1 bottle	—	—
		R50AF-2	125 mL	—	1 bottle	5 bottles
Blocker A Kit	RT	R93AA-2	250 mL	1 kit	1 kit	5 kits
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles

RT = room temperature
dash (—) = not applicable
‡Plates and detection antibody vials may be labeled as VEGF.

Additional Materials and Equipment Required

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid-handling equipment suitable for dispensing 10 µL to 150 µL/well into a 96-well microtiter plate
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) or phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Mixing or substituting reagents from different sources or kit lots is not recommended. Lot information is provided in the lot-specific COA
- Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs
- Bring frozen diluents to room temperature in a 20–26 °C water bath before use. If a controlled water bath is not available, thaw at room temperature. Diluents may also be thawed overnight at 2–8 °C. Thaw other reagents on wet ice and use them immediately.
- Prepare calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates need not be shielded from light except for direct sunlight.
- Avoid bubbles in wells during all pipetting steps, as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
- Plate shaking should be vigorous, with a rotary motion between 500–1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
- Tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seal before reading the plate.
- Read buffer should be at room temperature (20–26 ºC) before adding it to the plate.
- Do not shake the plate after adding the read buffer.
- Keep time intervals consistent between adding the read buffer and reading the plate to improve inter-plate precision. It is recommended that an MSD instrument be prepared to read a plate before adding Read Buffer. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Reagent Preparation

Bring all the reagents to room temperature and refer to the Best Practices section (page 7) before beginning the protocol. Thaw the stock calibrator on ice. **Important:** Upon the first thaw, aliquot Diluent 37 into suitably sized aliquots before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Standards

MSD supplies a blended calibrator for the Kidney Injury Panel 3 (human) Kit at a 20-fold higher concentration than the recommended highest calibrator. We recommend a 7-point standard curve with 4-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep it on ice, then add it to the diluent at room temperature to make the standard curve solutions. For the actual concentration of each calibrator in the blend, refer to the certificate of analysis (COA) supplied with the kit or available at www.mesoscale.com.

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of stock calibrator to 285 µL of Diluent 37. Mix well.
2) Prepare the next standard by transferring 60 µL of the highest standard to 180 µL of Diluent 37. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 37 as the blank.

Dilute Samples

For human urine samples, MSD recommends a 10-fold dilution in Diluent 37; however, you may adjust dilution factors for the sample set under investigation. We recommend diluting human serum samples 10-fold for all analytes except clusterin, which requires a greater than 10-fold dilution. To dilute a sample 10-fold, add 20 µL of sample to 180 µL of Diluent 37. The kit includes diluent sufficient for running samples in duplicates. Additional diluent can be purchased at www.mesoscale.com.

Prepare Detection Antibody Solution

MSD provides each detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For one plate, combine the following detection antibodies and add to 2,640 µL of Diluent 37.

- ❑ 60 µL of 50X SULFO-TAG Anti-human Calbindin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-human Clusterin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-human HAVCR1/KIM-1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-human Osteoactivin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-human TFF3 Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-human VEGF-A Antibody

Note: You may omit detection antibody for any analyte not being measured; add 60 µL of Diluent 37 for each omitted antibody.

Prepare Wash Buffer

MSD provides 100 mL of MSD Wash Buffer as a 20X stock solution. The working solution is 1X. PBS-T can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For one plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

Note: You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Note: Follow Reagent Preparation (page 8) before beginning this assay protocol.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 2: Wash and Add Sample

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  Note: You may prepare detection antibody solution during incubation.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  Note: You may prepare read buffer during incubation.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Analysis of Results

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

Typical Data

The following standard curves (Figure 2) illustrates the dynamic range of the assay. Actual signals will vary. The best measurement of unknown samples will be achieved by generating a standard curve for each plate using a minimum of two replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.

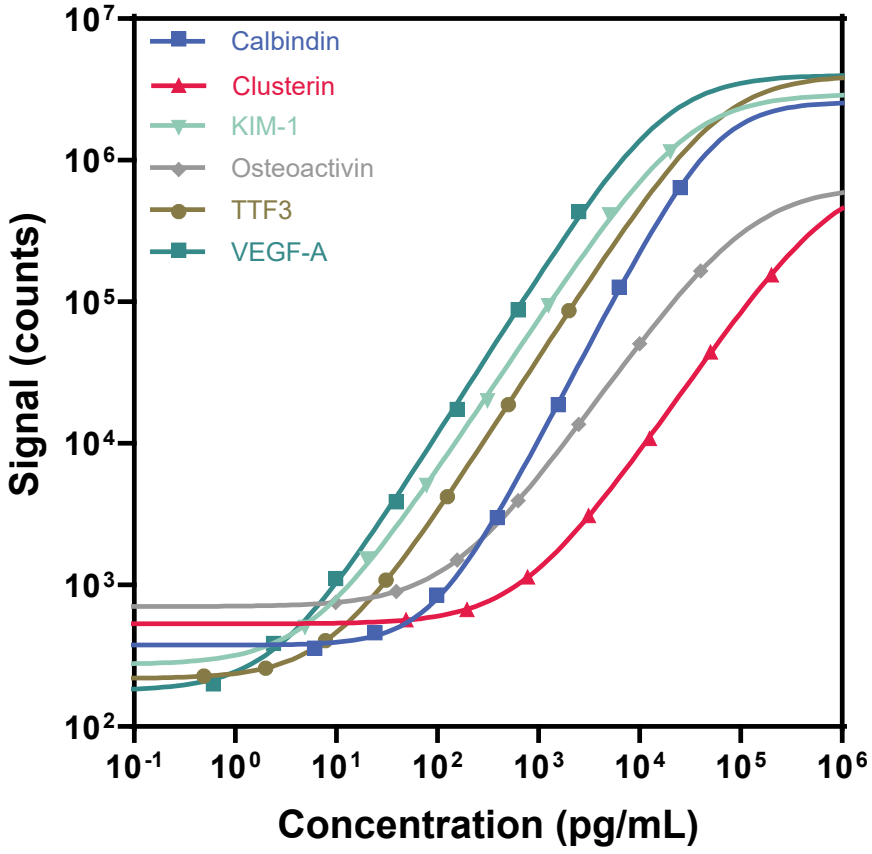

Figure 2. Typical calibration curves for the Kidney Injury Panel 3 (human) Kit.

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated from nine kit lots (Table 2).

Table 2. LLOD for each analyte in the Kidney Injury Panel 3 (human) Kit

	Calbindin	Clusterin	HAVCR1/KIM-1	Osteoactivin‡	TFF3‡	VEGF-A
Median LLOD (pg/mL)	7.9	32	1.6	12	2.9	0.17
LLOD Range (pg/mL)	6.4–8.8	22–160	1.3–2.3	7.9–20	1.5–6.6	0.13–0.24

Precision

Human urine-based control samples with high, medium, and low levels of each analyte were measured using a minimum of two replicates on six runs over two days. Average intra-run %CV is the average %CV of the control replicates on an individual run. Inter-run %CV is the variability of controls across six runs (Table 3).

Table 3. Intra-run and Inter-run %CVs for each analyte in the Kidney Injury Panel 3 (human) Kit

	Control	Runs	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV
Calbindin	High	6	10,300	4.9	4.5
	Mid	6	1,390	4.5	4.5
	Low	6	172	3.5	3.2
Clusterin	High	6	28,900	5.4	5.2
	Mid	6	5,780	12.4	11.4
	Low	6	771	8.7	16.2
HAVCR1/KIM-1	High	6	16,700	8.0	7.9
	Mid	6	2,360	3.6	3.3
	Low	6	107	2.5	3.9
Osteoactivin	High	6	2,690	5.6	6.0
	Mid	6	230	5.0	5.6
	Low	6	1,020	6.7	6.6
TFF3	High	6	173	5.2	5.6
	Mid	6	29	4.1	4.2
	Low	6	1,050	3.5	4.2
VEGF-A	High	6	119	3.6	5.0
	Mid	6	10	6.0	5.6
	Low	6	10,300	4.9	4.5

Dilution Linearity

To assess linearity, normal human urine samples were diluted 4-fold, 8-fold, 16-fold, 32-fold, and 64-fold before testing. Percent recovery at each dilution was calculated by dividing the measured concentration by the expected concentration, i.e., the concentration of the previous dilution. The average percent recovery shown below was calculated from samples with values above the LLOD (Table 4).

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} \text{x } 100$$

Table 4. Analyte percent recovery at various dilutions in each sample type

		Calbindin		Clusterin		HAVCR1/KIM-1	
Sample Type	Fold Dilution	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range
Urine (N = 8)	4	124	113–137	77	63–88	89	85–94
	8	110	100–128	86	73–95	91	85–96
	16	109	104–117	91	84–96	96	93–99
	32	106	101–112	103	94–10	103	98–104
	64	111	101–119	95	91–99	99	95–101

		Osteoactivin		TFF3		VEGF-A	
Sample Type	Fold Dilution	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range
Urine (N = 8)	4	97	81–105	105	91–115	93	89–97
	8	90	81–99	74	62–81	96	91–104
	16	97	84–106	69	60–77	106	99–112
	32	91	88–94	71	68–74	108	101–120
	64	<LLOD	<LLOD	<LLOD	<LLOD	104	101–111

Note: Some assays showed significant matrix effects, which can be minimized by higher sample dilution.

Spike Recovery

Normal human urine samples were diluted 20-fold and then spiked with calibrators at multiple levels throughout the range of the assay. The average percent recovery shown below was calculated from samples with values above the LLOD (Table 5).

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} \text{x}\ 100$$

Table 5. Spike and recovery measurements of different sample types in the Kidney Injury Panel 3 (human) Kit

	Calbindin			Clusterin			HAVCR1/KIM-1		
	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range
Urine (N = 8)	78	95	87–102	625	92	75–106	31	111	101–134
	313	87	81–94	2,500	95	86–104	125	100	89–107
	1,250	87	80–93	10,000	102	94–109	500	87	83–93
	5,000	89	80–97	40,000	103	99–108	2,000	96	89–104
	20,000	86	77–92	160,000	103	96–113	8,000	111	107–118

	Osteoactivin			TFF3			VEGF-A		
	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range	Spike Conc. (pg/mL)	Average% Recovery	%Recovery Range
Urine (N = 8)	78	86	81–96	6.3	104	93–113	31	7.8	101
	313	83	72–92	25	94	89–100	125	31	91
	1,250	79	70–87	100	95	89–100	500	125	91
	5,000	83	75–90	400	99	94–106	2,000	500	98
	20,000	79	73–86	1,600	94	92–97	8,000	2,000	106

Specificity

To assess the specificity of the detection antibodies, the Kidney Injury Panel 3 (human) kit was run using blended calibrators with individual detection antibodies and using blended detection antibodies with individual calibrators (6.0 ng/mL Calbindin; 25.0 ng/mL Clusterin; 4.0 ng/mL HAVCR1/KIM-1; 15.0 ng/mL Osteoactivin; 0.3 ng/mL TFF3; and 0.5 ng/mL VEGF-A). No significant cross-reactivity (<1%) was observed.

Stability

Kit components were tested for freeze-thaw stability. Results (not shown) demonstrated that blended calibrator and controls can go through five freeze-thaw cycles without affecting assay performance.

Tested Samples

Normal Samples

Normal and disease samples (both urine and serum) were diluted 10-fold and tested with the Kidney Injury Panel 3 (human) kit. Median and range of concentrations for each sample set are displayed below (Table 6). Concentrations are corrected for sample dilution.

Clinical information associated with normal and kidney disease samples was not available

Table 6. Intra-run and Inter-run %CVs for each analyte in the Kidney Injury Panel 3 (human) Kit

Sample Type	Statistic	Calbindin	Clusterin	HAVCR1/KIM-1	Osteoactivin	TFF3	VEGF-A
Normal Urine (N = 35)	Median (pg/mL)	4.5	24	0.31	0.24	<LLOD	0.45
	Range (pg/mL)	<LLOD–13	<LLOD–200	<LLOD–2.2	<LLOD–0.60	<LLOD–0.53	<LLOD–1.4
	Samples above LLOD	34	33	34	34	15	34
Kidney Disease Urine (N = 15)	Median (pg/mL)	2.6	58	1.4	0.37	0.043	0.40
	Range (pg/mL)	0.61–15	2.3–253	0.083–3.7	0.18–1.1	<LLOD–2.6	0.19–0.83
	Samples above LLOD	15	15	15	15	9	15
Normal Serum (N = 15)	Median (pg/mL)	4.9	*	0.17	7.9	0.31	0.16
	Range (pg/mL)	2.0–8.0	*	0.11–0.26	5.6–18	0.17–0.51	0.098–0.21
	Samples above LLOD	15	*	15	15	15	15
Kidney Disease Serum (N = 15)	Median (pg/mL)	4.1	*	0.29	11	0.69	0.67
	Range (pg/mL)	2.9–6.4	*	0.19–0.83	7.6–17	0.41–2.3	0.15–4.0
	Samples above LLOD	15	*	15	15	15	15

*Sample signal exceeds the top of the standard curve at a 10-fold dilution signal. Clusterin testing in human serum requires > 10-fold dilution

Assay Components

Calibrators

The assay calibrator blend uses the following recombinant rat proteins (Table 7).

Table 7. Recombinant rat proteins used in the calibrator

	Expression System
Calbindin	*E. coli*
Clusterin	Murine cell line
HAVCR1/KIM-1	Murine cell line
Osteoactivin	Murine cell line
TFF3	*E. coli*
VEGF-A	Insect cell line

Antibodies

The antibody source species and types are described in Table 8.

Table 8. Antibody source species and types

	Source Species	
	Capture Antibody	Detection Antibody
Calbindin	Mouse monoclonal	Goat polyclonal
Clusterin	Mouse monoclonal	Goat polyclonal
HAVCR1/KIM-1	Goat polyclonal	Goat polyclonal
Osteoactivin	Goat polyclonal	Goat polyclonal
TFF3	Mouse monoclonal	Mouse monoclonal
VEGF-A	Mouse monoclonal	Mouse monoclonal

Summary Protocol

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare Blocker A solution
- ❑ Prepare calibration solutions in Diluent 37 using the supplied calibrator.
  - Dilute the stock calibrator 20-fold in Diluent 37.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute samples 10-fold in Diluent 37 before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 37.
- ❑ Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 2: Wash and Add Sample

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of sample (standards or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL of 2X Read Buffer T to each well.
- ❑ Analyze the plate on an MSD instrument.

Plate Diagram

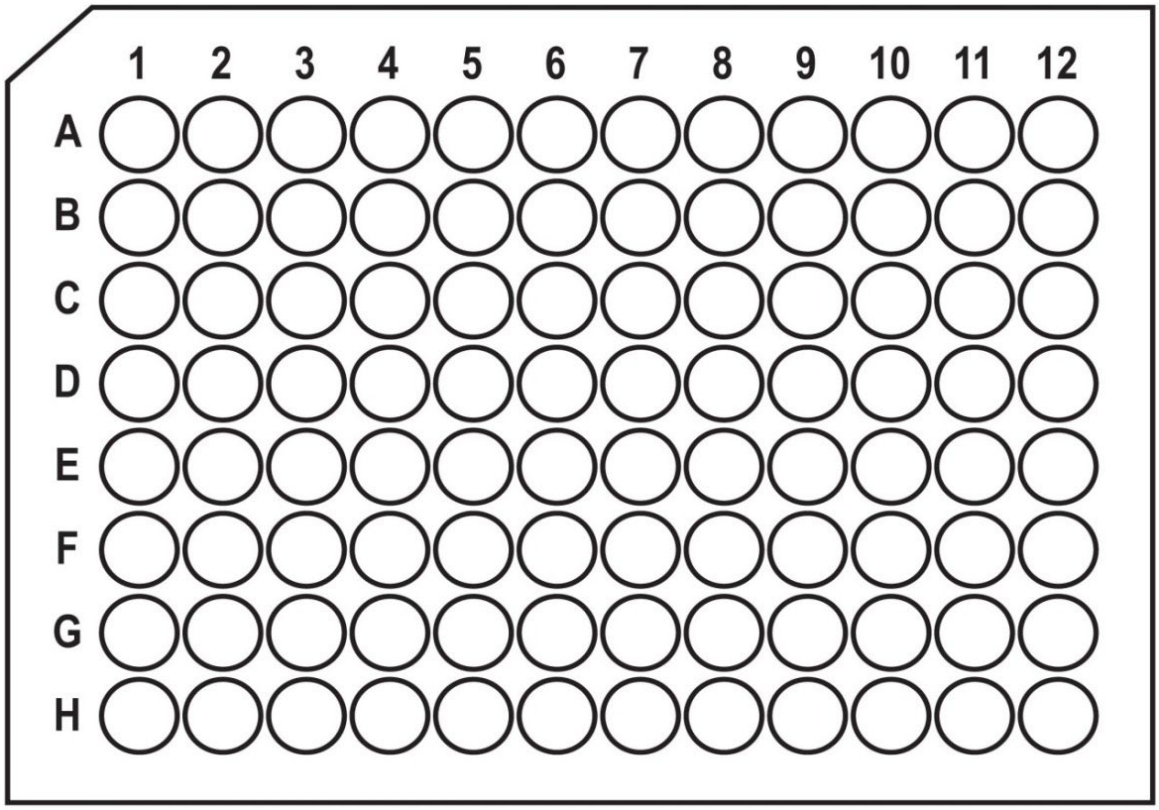

Figure 3. Plate diagram.