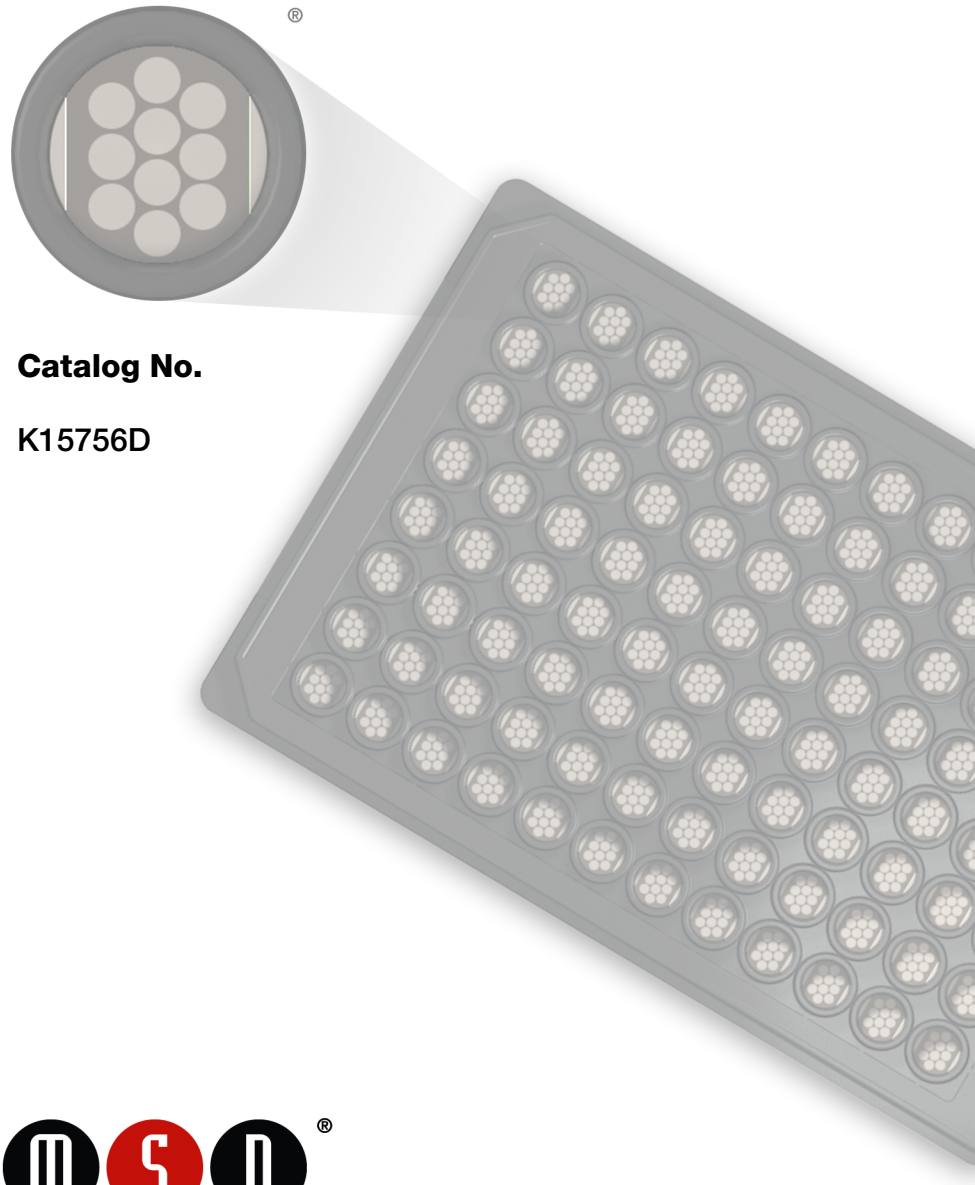
MSD® MULTI-SPOT Assay System

Kidney Injury Panel 3 (human) Kit

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

T-PLEX®



Catalog No.

Multiplex Kit

K15756D

MSD®

MSD Toxicology Assays

Kidney Injury Panel 3 (human) Kit

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

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

Table of Contents

Contact Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Technical Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 Attn: Technical Support
Email: ScientificSupport@mesoscale.com

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 T-PLEX® 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

MESO SCALE DISCOVERY® 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 T-PLEX 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.

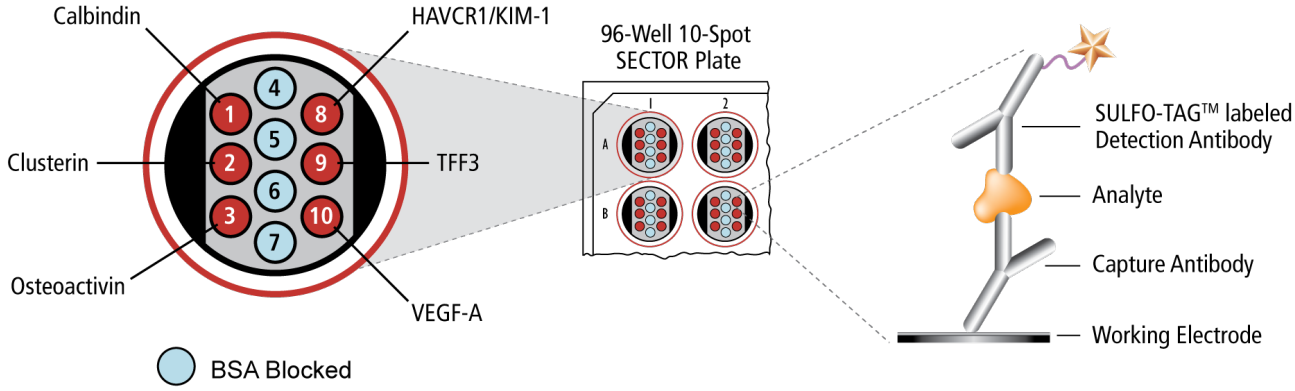


Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Standard Workflow for T-PLEX Kidney Injury Panel 3 (human) Kit

An overview of the steps in the T-PLEX Kidney Injury Panel 3 (human) Kit workflow with incubation durations is outlined below. This is an overview for planning purposes; for the full workflow, see *Protocol* on page 8.

Step	Incubation
Add Blocker A solution	30 min.
Wash and add sample	2 hr.
Wash and add detection antibody solution	2 hr.
Add read buffer	No incubation
Read plate	Read time is dependent on instrument model

Materials and Equipment

This section lists the components provided with the T-PLEX Kidney Injury Panel 3 (human) Kit.

Reagents Supplied

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

Reagent	Storage	Catalog No.	Size	Quantity Supplied		
				1 Plate	5 Plates	25 Plates
MULTI-SPOT® 96-Well 10-Spot Kidney Injury Panel 3 (human) Plate‡, 2	2–8 °C	N05189A-1	1 plate	1	5	25
Human Calbindin Antibody (50X)	2–8 °C	D21KS-2	75 µL	1 vial	—	—
		D21KS-3	375 µL	—	1 vial	5 vials
Human Clusterin Antibody (50X)	2–8 °C	D21HX-2	75 µL	1 vial	—	—
		D21HX-3	375 µL	—	1 vial	5 vials
Human HAVCR1/KIM-1 Antibody (50X) 2	2–8 °C	D21JH-2	75 µL	1 vial	—	—
		D21JH-3	375 µL	—	1 vial	5 vials
Human Osteoactivin Antibody (50X)	2–8 °C	D21KT-2	75 µL	1 vial	—	—
		D21KT-3	375 µL	—	1 vial	5 vials
Human TFF3 Antibody (50X)	2–8 °C	D21KU-2	75 µL	1 vial	—	—
		D21KU-3	375 µL	—	1 vial	5 vials
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
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—
		R60AM-2	90 mL	—	1 bottle	5 bottles

RT = room temperature
dash (—) = not applicable
‡Plates and detection antibody vials may be labeled as VEGF.
2Plates and detection antibody vials may be labeled as KIM-1.

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid-handling equipment suitable for dispensing 10 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 700–1,000 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

Assay Components

The assay calibrator blend and antibody source species and types are described in Table 2 and Table 3.

Calibrators

Table 2. *Recombinant rat proteins used in the calibrator*

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

Table 3. *Antibody source species and types*

Analyte	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

Safety

Use safe laboratory practices. Wear appropriate personal protective equipment to include 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.

Protocol

This protocol is for 96-well plates.

Best Practices

Read this product insert in its entirety before use. In addition, adhere to the following best practices:

Reagent Preparation

Do Not Mix Lots	Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
Thaw Diluents	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	Thaw other reagents on wet ice and use them immediately.

Reagent Handling

Prepare in Polypropylene Tubes	Prepare calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
Protect Reagents from Light	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 During Pipetting	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	Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the bottom of the wells with the pipette tip when pipetting into the MSD plate.

Plate Handling

Protect Plate from Sunlight	Protect plates from direct sunlight.
Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 700–1,000 rpm.
Incubation Temperature	Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs.
Tap Plate	Tap the plate on a paper towel to remove residual fluid after washing.
Incubation Extension	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.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate.
Read Buffer at Room Temperature	Ensure that the read buffer is at room temperature (20–26 °C) before adding to the plate.
Do Not Shake Plate	Do not shake the plate after adding read buffer.
Time Intervals	Keep time intervals consistent between adding the read buffer and reading the plate to improve inter-plate precision. Prepare the MSD instrument to read a plate before adding read buffer. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
Results Above Curve	If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Reagent Preparation

Bring reagents to room temperature and refer to *Best Practices* on page 8 before beginning the protocol. Thaw the stock calibrator on ice.

IMPORTANT

Upon 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 T-PLEX 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 (Diluent 37).

To prepare 7 standard solutions plus a zero calibrator blank for up to 2 replicates:

1. Thaw the stock calibrator and keep it on ice.
2. Add calibrator to the Diluent 37 at room temperature to make the standard curve solutions according to the scheme below. Mix well between each step.

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Diluent 37 (µL)	Total volume (µL)
1	1	Stock calibrator	10	190	200
2	2	From tube 1	50	150	200
3	3	From tube 2	50	150	200
4	4	From tube 3	50	150	200
5	5	From tube 4	50	150	200
6	6	From tube 5	50	150	200
7	7	From tube 6	50	150	200
8 (zero calibrator)	8	—	0	200	200

Dash (—) = not applicable

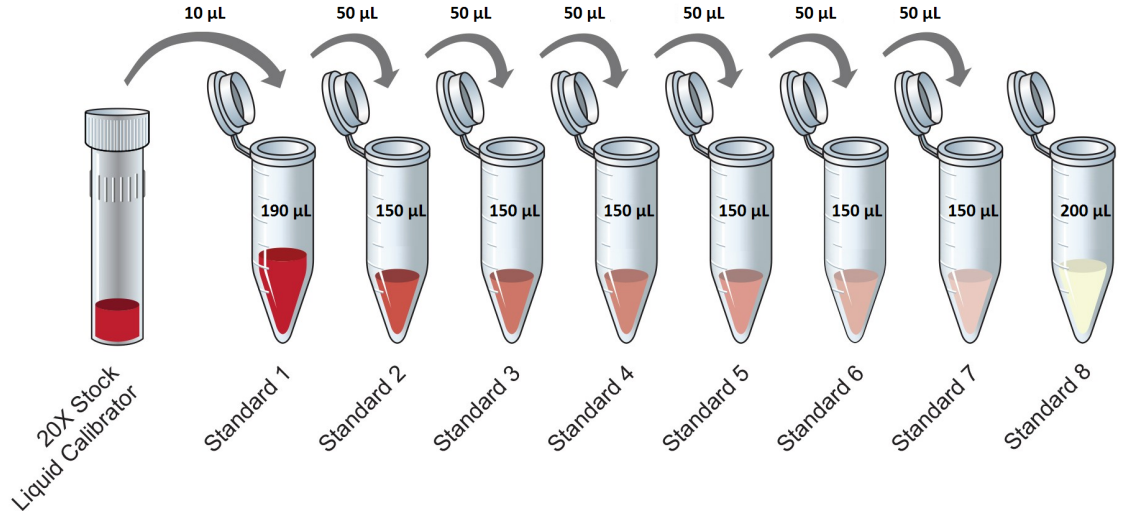


Figure 2. *Dilution scheme for preparation of calibrator standards.*

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.

Dilute Samples

MSD recommends the following dilutions:

- For human urine samples, dilute 10-fold in Diluent 37.
- For human serum samples, dilute 10-fold in Diluent 37 for all analytes except clusterin, which requires a >10-fold dilution.
- You may adjust dilution factors for the sample set under investigation.

To dilute a sample 10-fold, combine:

- ❑ 20 µL of sample
- ❑ 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 concentration is 1X.

For 1 plate, add the following detection antibodies to Diluent 37 for a total of 3,000 µL:

- ❑ 2,640 µL Diluent 37
- ❑ 60 µL of 50X SULFO-TAG™ Human Calbindin Antibody
- ❑ 60 µL of 50X SULFO-TAG Human Clusterin Antibody
- ❑ 60 µL of 50X SULFO-TAG Human HAVCR1/KIM-1 Antibody
- ❑ 60 µL of 50X SULFO-TAG Human Osteoactivin Antibody
- ❑ 60 µL of 50X SULFO-TAG Human TFF3 Antibody
- ❑ 60 µL of 50X SULFO-TAG Human VEGF-A Antibody

> **i** You may omit detection antibody for any analyte not being measured. In that case, add 60 µL of Diluent 37 for each omitted antibody.

Prepare Wash Buffer

MSD provides 100 mL of MSD Wash Buffer (20X) stock solution. The working solution is 1X.

> **i** PBS-T can be used instead of MSD Wash Buffer.

For one plate, combine:

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

Read Buffer

MSD GOLD Read Buffer B is provided ready to use. Do not dilute.

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.

Assay Protocol

This section describes the assay protocol. Complete *Reagent Preparation* on page 9 before beginning this assay protocol.

STEP 1: Add Blocker A Solution

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

STEP 2: Wash and Add Sample

❑ 2. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 3. Add 50 µL of sample (standards or unknowns) to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

i You can prepare detection antibody solution during the incubation.

STEP 3: Wash and Add Detection Antibody Solution

❑ 4. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 5. Add 25 µL of 1X Detection Antibody Solution to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 4: Wash and Read

❑ 6. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 7. Add 150 µL of MSD GOLD Read Buffer B to each well.

❑ 8. Read the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Analysis of Results

When analyzing results, note the following:

- 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 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.

Assay Characteristics

Typical Data

The following standard curves (Figure 3) illustrate the dynamic range of the assay. Actual signals will vary. The best measurement of unknown samples is 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. The lot-specific COA is supplied with the kit and is available for download at www.mesoscale.com.

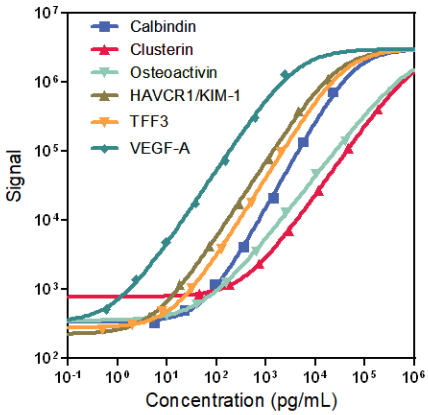


Figure 3. Typical calibration curves for the T-PLEX Kidney Injury Panel 3 (human) Kit.

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 run on 10-spot plates (Table 4).

Table 4. LLOD for each analyte in the T-PLEX Kidney Injury Panel 3 (human) Kit.

Analyte	Median LLOD (pg/mL)
Calbindin	15.2
Clusterin	52.6
Osteoactivin	10.8
HAVCR1/KIM-1	1.77
TFF3	3.78
VEGF-A	0.156

Precision

Commercially sourced 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 on 10-spot plates. 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 5).

Table 5. *Intra-run and inter-run %CVs for each analyte in the T-PLEX 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

Tested Samples

Commercially sourced normal and disease samples (both urine and serum) were diluted 10-fold and tested with the T-PLEX Kidney Injury Panel 3 (human) Kit (7-spot plates). Median and range of concentrations for each sample set are displayed below (Table 6). Concentrations are corrected for sample dilution.

Table 6. Concentration statistics (median, range, samples above LLOD) by sample type for each analyte in the T-PLEX Kidney Injury Panel 3 (human) Kit.

Sample Type	Statistic	Calbindin	Clusterin	HAVCR1/KIM-1	Osteoactivin	TFF3	VEGF-A
Normal Urine (N = 35)	Median (ng/mL)	4.5	24	0.31	0.24	<LLOD	0.45
	Range (ng/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 (ng/mL)	2.6	58	1.4	0.37	0.043	0.40
	Range (ng/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 (ng/mL)	4.9	*	0.17	7.9	0.31	0.16
	Range (ng/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 (ng/mL)	4.1	*	0.29	11	0.69	0.67
	Range (ng/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 > 1,000-fold dilution.

Dilution Linearity

To assess linearity, commercially sourced 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 7).

$$\%\ recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 7. Analyte percent recovery at various dilutions in each sample type using 7-spot plates

		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

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

Spike Recovery

Commercially sourced normal human urine samples were diluted 20-fold and then spiked with calibrators at multiple levels throughout the range of the assay using 7-spot plates. The average percent recovery shown below was calculated from samples with values above the LLOD (Table 8).

$$\%\ recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 8. Spike and recovery measurements of different sample types in the T-PLEX 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 T-PLEX Kidney Injury Panel 3 (human) Kit was run on 7-spot plates 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.

Additional Information

Appendix A: Recommended Plate Washer Parameters

We recommend creating a new program for your automated plate washer with the optimal settings before starting your assay. Example settings for a typical (MSD-recommended) wash program are shown below for a common plate washer (Biotek Model 405 LS,Table 9).

Table 9. *Parameters for customized programs on the Biotek 405 LS microplate washer*

Wash Program Parameters		Typical Wash Program Settings
	Plate type	96
CYCLES	Wash cycles	3
ASPIRATION	Aspirate Type	TOP
	Travel Rate	1 (4.1% 1.0 mm/second)
	Aspirate Delay	0500 milliseconds
	Aspirate X-Position	-35
	Aspirate Y-Position	-35
	Aspirate Height	22
	Secondary Aspirate?	NO
DISPENSE	Dispense Rate	05
	Dispense Volume	0300 µL/well
	Vacuum Delay Volume	0300 µL/well
	Dispense X-Position	00 (0.000 mm)
	Dispense Y-Position	00 (0.000 mm)
	Dispense Height	120 (15.245 mm)
OPTS PRE	Wash Pre dispense?	NO
	Bottom Wash?	NO
MIDCYC	Wash Shake?	NO
	Wash Soak?	NO
	Home Carrier?	NO
	Between Cycle Pre Dispense?	NO
POST	Final Aspirate?	YES
	Aspirate Type	TOP
	Travel Rate	3
	Final Aspirate Delay	0500 milliseconds
	Final Aspirate X-Position	-35 (1.600 mm)
	Final Aspirate Y-Position	-35 (1.600 mm)
	Final Aspirate Height	22
	Secondary Aspirate?	YES
	Final Aspirate Secondary X-Position	35 (1.600 mm)
	Final Aspirate Secondary Y-Position	35 (1.600 mm)
	Final Aspirate Secondary Height	22

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.

STEP 1: Add Blocker A Solution

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

STEP 2: Wash and Add Sample

❑ 2. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 3. Add 50 µL of sample (standards or unknowns) to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 3: Wash and Add Detection Antibody Solution

❑ 4. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 5. Add 25 µL of 1X Detection Antibody Solution to each well.
Seal the plate with an adhesive plate seal.
Incubate 2 hours at room temperature with vigorous shaking (500–1,000 rpm).

STEP 4: Wash and Read

❑ 6. Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ 7. Add 150 µL of MSD GOLD Read Buffer B to each well.

❑ 8. Read the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Plate Diagram

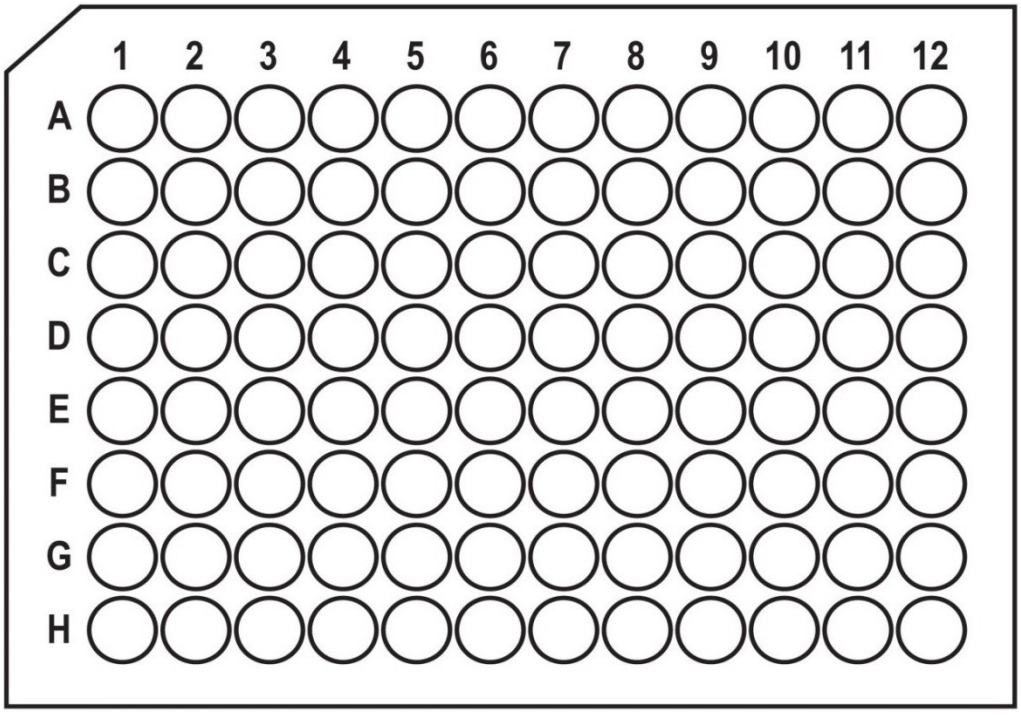


Figure 4. Plate diagram.

Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

Figure 5. Recommended plate layout for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.

Catalog Numbers

Table 10. Catalog numbers associated with the T-PLEX Kidney Injury Panel 3 (human) Kit

Kit Name	SECTOR Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit
T-PLEX Kidney Injury Panel 3 (human) Kit	K15756D-1	K15756D-2	K15756D-4

Table 11. Instrument compatibility for plate type

Plate Type	Instrument Compatibility
SECTOR™ Plate	MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, MESO QuickPlex SQ 120MM

Ensure the plate type is compatible with your MSD instrument.