

# Meso Scale Discovery<sup>®</sup>

## MULTI-ARRAY<sup>®</sup> Assay System

### Rat Clusterin Assay Kit

1-Plate Kit

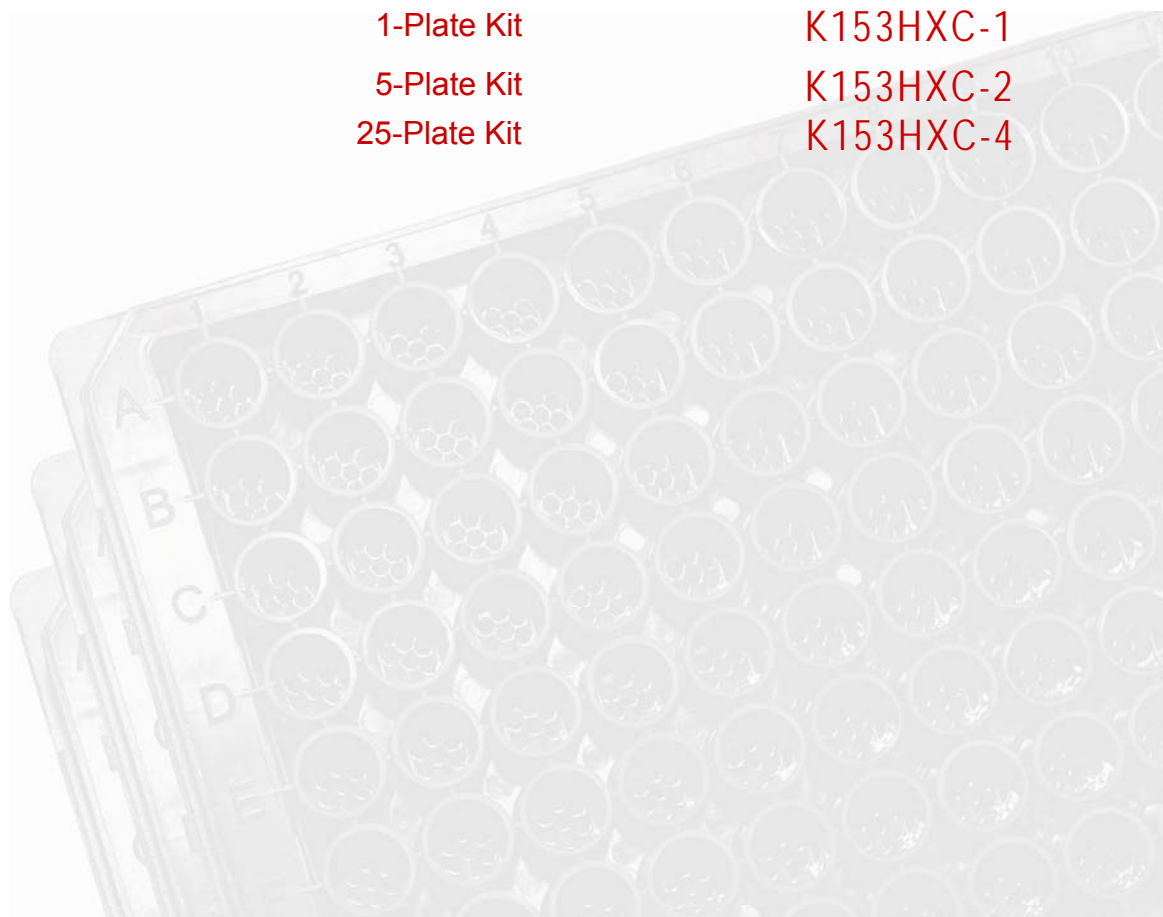
K153HXC-1

5-Plate Kit

K153HXC-2

25-Plate Kit

K153HXC-4



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# MSD Toxicology Assays

## Rat Clusterin Assay Kit

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

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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## Ordering Information

ordering information

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# Introduction

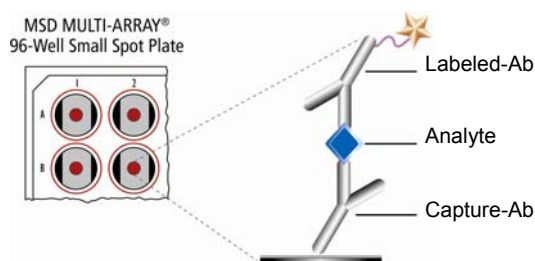
introduction

**Clusterin** is a glycoprotein that is found in most mammalian tissues. The localized over-expression of clusterin at sites of tissue damage or stress implicates that clusterin acts as a molecular chaperone displaying cytoprotective characteristics. In addition, the marked induction of clusterin in several renal disease states suggest that clusterin may be a putative biochemical marker of kidney damage and disease.

## Principle of the Assay

principle of the assay

MSD<sup>®</sup> toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and panel formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a panel assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Rat Clusterin Assay detects clusterin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with clusterin antibody. The user adds the sample and a solution containing labeled detection antibody—anti -rat Clusterin labeled with an electrochemiluminescent compound, MSD SULFO-TAG<sup>™</sup> label—over the course of one or more incubation periods. Clusterin in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR<sup>®</sup> instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of clusterin present in the sample.



**Figure 1.** Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

# Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K153HXC-1	K153HXC-2	K153HXC-4
MULTI-ARRAY 96-well Rat Clusterin Plate(s) L453HXA-1	2-8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat Clusterin Antibody (50X) <sup>1</sup>	2-8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat Clusterin Calibrator (20X) 4000 ng/mL	≤ -70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
10% Triton X-100	2-8°C	1 vial (1.0 mL)	1 vial (1.0 mL)	5 vials (1.0 mL ea)
Diluent 5 R52BA-5 (25 mL)	≤ -10°C	1 bottle (25 mL)	3 bottles (25 mL ea)	15 bottles (25 mL ea)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

## Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

## Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

<sup>1</sup> Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

# VI

## Reagent Preparation

### reagent preparation

**Important:** The Rat Clusterin Calibrator **stock** solution should be thawed and kept on ice during all pipetting steps. The diluted Calibrators should be kept at room temperature prior to addition to the plate.

Bring all other reagents to room temperature.

**Important:** Upon first thaw, separate Diluent 5 into aliquots appropriate to the size of your assay needs. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

### Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

### Prepare Assay Diluent

Prepare a solution of 0.4% Triton X-100 in Diluent 5. For one plate, mix 2.88 mL of Diluent 5 with 0.12 mL of 10% Triton X-100. For each plate, 25  $\mu$ L per well of this solution will be required.

### Prepare Calibrator and Control Solutions

Calibrator for the Rat Clusterin Assay is supplied at 20-fold higher concentration than the recommended highest Calibrator. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Clusterin conc. (ng/mL)	Dilution Factor
Stock Cal. Vial	4000	
STD-01	200	20
STD-02	66.7	3
STD-03	22.2	3
STD-04	7.41	3
STD-05	2.47	3
STD-06	0.82	3
STD-07	0.27	3
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

- 1) Prepare the highest Calibrator by adding 12  $\mu$ L of the Calibrator stock vial to 228  $\mu$ L of Diluent 5 (**NOT** containing Triton X-100) (20-fold dilution).
- 2) Prepare the next Calibrator by transferring 80  $\mu$ L of the diluted Calibrator to 160  $\mu$ L of Diluent 5. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) Reserve 160  $\mu$ L of Diluent 5 to be used as zero Calibrator.

Calibrators should be prepared no more than 30 minutes before use.

## **Dilution of Samples**

Some rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. If this is the case, Diluent 5 should be used to dilute samples. A 5-fold dilution of urine samples is recommended. Depending on the sample set under investigation, higher or lower dilution factors may be necessary. For kidney tissue lysates or homogenates, 10–500-fold dilution may be required.

## **Prepare Detection Antibody Solution**

The Detection Antibody is provided as a 50X stock of Anti-rat Clusterin Antibody. The working Detection Antibody Solution should contain 1X as final concentration. For each plate used, dilute 60  $\mu$ L of the stock Detection Antibody stock into a final volume of 3 mL of Diluent 5.

## **Prepare Read Buffer**

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

## **Prepare MSD Plate**

This plate has been pre-coated with antibody as shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

# VII Assay Protocol

## assay protocol

- 1. Addition of Blocker A Solution:** Dispense 150  $\mu$ L of Blocker A Solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
- 2. Wash and Addition of Sample or Calibrator:** Wash the plate 3X with PBS-T. First, dispense 25  $\mu$ L of Assay Diluent (Diluent 5 + 0.4% Triton X-100) into each well of the MSD plate. Then, dispense 25  $\mu$ L of sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 3. Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25  $\mu$ L of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
- 4. Wash and Read:** Wash the plate 3X with PBS-T. Add 150  $\mu$ L of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

### Notes

*Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.*

*Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.*

# VIII Analysis of Results

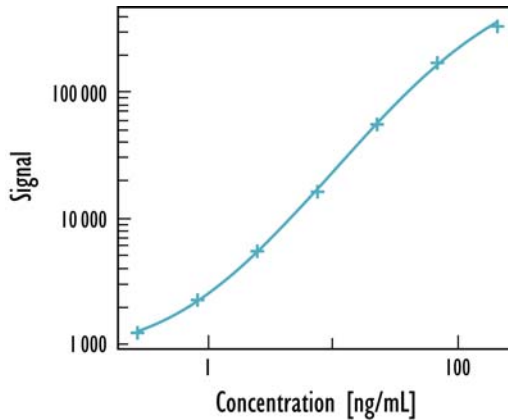
## analysis of results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench<sup>®</sup> analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a  $1/Y^2$  weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

# IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



Conc. (ng/mL)	Mean	%CV
0	660	6.2
0.27	1217	5.9
0.82	2232	5.4
2.47	5470	2.8
7.41	16359	4.1
22.2	55620	4.9
66.7	172202	3.6
200	338824	3.4

# X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero Calibrator.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

	Clusterin (ng/mL)
LLOD	0.017
LLOQ	0.82
ULOQ	150

# XI Precision

## precision

High, mid, and low controls were made by spiking recombinant protein into stock Calibrator diluent. The controls were run in quadruplicate on each of 10 plates run across three days. The controls were run at a 10-fold dilution.

	Control	Plates	Average Conc. (ng/mL)	Intra-Plate	Inter-Plate
				Average %CV	%CV
Clusterin	High	10	139	7.27	10.4
	Mid	10	17.5	4.30	6.06
	Low	10	1.90	4.80	6.57

# XII Spike Recovery

## spike recovery

Rat urine samples were spiked with clusterin standard at multiple levels throughout the range of the assay. The samples were diluted either 5-fold or 10-fold and then spiked with Clusterin Standard at the levels indicated in the below table. Control sample 1 is a urine sample from an individual rat, and control sample 2 is a pooled urine sample.

% Recovery = measured / expected x 100

Sample	Spike level (ng/mL)	Conc. (ng/mL)	% CV	% Recovery
Control sample 1, 5-fold dilution	124	161	2.49	129
	14.1	17.1	2.30	115
	0.57	1.54	5.34	112
	0	0.8	5.60	
Control sample 2, 5-fold dilution	124	118	4.50	94
	14.2	13.9	6.10	93
	0.67	1.44	5.86	97
	0	0.8	8.70	
Control sample 2, 10-fold dilution	124	119	2.68	95
	14.6	13.9	3.11	93
	1.08	1.44	0.64	96
	0	0.4	1.50	

# XIII Linearity

linearity

Serial dilutions of a several rat urine samples were tested to assess linearity. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected). Measurements below the assay LLOQ are shown in grey.

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

Sample	Dilution Factor	Conc. (ng/mL)	Conc. % CV	% Recovery
Sample 1	5	43.6	3.22	
	10	55.9	2.63	128
	20	64.4	0.48	115
	40	67.0	1.98	104
	80	64.5	3.33	96
Sample 2	5	31.9	1.54	
	10	36.6	3.31	115
	20	41.2	1.39	112
	40	42.8	3.95	104
	80	42.8	13.6	100
Sample 3	5	12.9	4.69	
	10	14.2	2.54	111
	20	14.3	2.22	101
	40	14.6	5.42	102
	80	15.8	6.46	108
Sample 4	5	103	2.98	
	10	141	2.35	138
	20	187	3.48	132
	40	216	1.74	116
	80	240	1.17	111
Sample 5	5	70.5	2.46	
	10	94.4	1.84	134
	20	126	2.04	133
	40	139	0.64	111
	80	158	2.22	113

# XIV Samples

s a m p l e s

The MSD Rat Clusterin Assay is designed for use with rat urine samples.

Urine samples from control and treated rats were tested for Clusterin. Treated animals were dosed with nephrotoxicants prior to sample collection. Samples were assayed at 5X dilution, and some were rerun at higher dilution ratio to get them in range. In the table below, measurements in gray were less than the dilution-adjusted LLOQ of 4.1 ng/mL.

Sample #	Type	Dilution Corrected Conc. (ng/mL)	Sample #	Type	Dilution Corrected Conc. (ng/mL)
1	Control	8.4	18	Control	6.5
2	Control	1.8	19	Treated	9.0
3	Control	4.2	20	Treated	469
4	Control	8.3	21	Treated	270
5	Control	9.3	22	Treated	591
6	Control	4.0	23	Treated	464
7	Control	6.6	24	Treated	1428
8	Control	7.3	27	Treated	111
9	Control	7.3	28	Treated	31.0
10	Control	3.9	29	Treated	83.0
11	Control	2.5	30	Treated	84.9
12	Control	12.3	34	Treated	356
13	Control	0.8	35	Treated	143
14	Control	1.0	36	Treated	213
15	Control	1.6	37	Treated	230
16	Control	9.8	38	Treated	231
17	Control	11.0			

# XV Calibrator

c a l i b r a t o r

Recombinant rat clusterin was calibrated against an internal control and diluted to a final concentration of 4000 ng/mL to make Rat Clusterin Calibrator.

# XVI Reference

r e f e r e n c e

Rached, E., Hoffman, D., Blumbach, K., Weber, K., Dekant, W., Mally, A. (2008) *Evaluation of Putative Biomarkers of Nephrotoxicity after Exposure to Ochratoxin A in vivo and in vitro*. Toxicol Sci. Feb 27:[Epub ahead of print]

## Summary Protocol

### MSD 96-well MULTI-ARRAY Rat Clusterin Assay Kit

MSD provides this summary protocol for your convenience.  
Please read the entire detailed protocol prior to performing the Rat Clusterin Assay.

#### Step 1 : Sample and Reagent Preparation

The Rat Clusterin Calibrator solution should be thawed and kept on ice during all pipetting steps. The diluted Calibrators should be kept at room temperature prior to addition to the plate. Bring appropriate diluents and plates to room temperature.

If necessary, samples should be diluted in Diluent 5.

Prepare Blocker A Solution.

Prepare Assay Diluent by diluting 10% Triton X-100 in Diluent 5 to a final concentration of 0.4% Triton X-100.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Diluent 5 without Triton X-100.
- Dilute the stock Calibrator 1:20 in Diluent 5, then perform a series of 3-fold dilution steps and a no Calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-rat Clusterin Antibody to 1X in 3.0 mL of Diluent 5 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X MSD Read Buffer T (with surfactant) with deionized water.

#### Step 2 : Add Blocker A Solution

Dispense 150  $\mu$ L/well Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

#### Step 3 : Wash and Add Sample or Calibrator

Wash plate 3X with PBS-T.

Dispense 25  $\mu$ L/well Assay Diluent (Diluent 5 + 0.4% Triton).

Dispense 25  $\mu$ L/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

#### Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25  $\mu$ L/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

#### Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150  $\mu$ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.



