MSD® MULTI-ARRAY Assay System

Rat Clusterin Kit

1-Plate Kit	K153MGC-1
5-Plate Kit	K153MGC-2
25-Plate Kit	K153MGC-4

MSD Toxicology Assays

Rat Clusterin Kit

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®

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

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Table of Contents

Introduction 4
Principle of the Assay 4
Reagents Supplied 5
Required Material and Equipment (not supplied) 5
Safety 5
Reagent Preparation 6
Protocol 8
Curve Fitting 9
Typical Data 9
Sensitivity 9
Precision 10
Dilution Linearity 10
Spike Recovery 10
Stability 11
Tested Samples 11
Assay Components 11
References 11
Summary Protocol 13
Plate Diagrams 15

Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

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 indicates that clusterin acts as a molecular chaperone displaying cytoprotective characteristics.[1] 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.[2]

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 Rat Clusterin kit is a sandwich immunoassay (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 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 provides 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 to provide a quantitative measure of analytes in the sample.

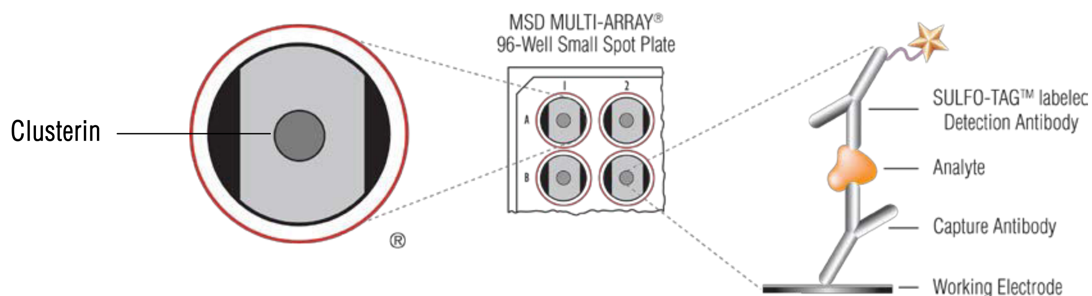

Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K153MGC-1	K153MGC-2	K153MGC-4
MULTI-ARRAY® 96-Well Small Spot Rat Clusterin Plate L453MGA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat Clusterin Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat Clusterin Calibrator (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 29 R50HA-3 (40 mL)	≤-10°C	1 bottle (40 mL)	2 bottles (40 mL ea)	10 bottles (40 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

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

Safety

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

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[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 29 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Standards

MSD supplies a calibrator for the Rat Clusterin assay at 20-fold higher concentration than the recommended highest standard. 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 on ice, then add to diluent at room temperature to make the standard curve solutions. To view the actual concentration of the calibrator, refer to the certificate of analysis (COA) supplied with the kit. You may also find a copy of the lot-specific COA at www.mesoscale.com by entering K153MGC in the search box.

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard by adding 15 µL of the stock calibrator to 285 µL of Diluent 29. Mix well.
2) Prepare the next standard by transferring 75 µL of the highest standard to 225 µL of Diluent 29. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 29 as the blank.

Dilute Samples

For urine samples, MSD recommends a 10-fold dilution in Diluent 29; however, you may adjust dilution factors for the sample set under investigation.

To dilute urine sample 10-fold, add 15 µL of sample to 135 µL of Diluent 29.

Prepare Detection Antibody Solution

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

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat Clusterin Antibody
- ❑ 2940 µL of Diluent 29

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

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

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

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

2. **Wash and Add Sample:** Wash the plate 3 times with 300 µL/well of 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 (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of 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 (300–1000 rpm) at room temperature.

   You may prepare diluted read buffer during incubation.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on an MSD instrument. No incubation in read buffer is required before reading the plate.

Notes

Shaking the plate typically accelerates capture at the working electrode.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

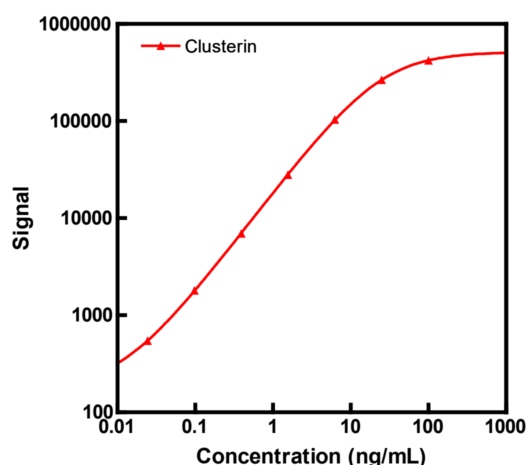
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate a standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the 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 curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.



	Clusterin	
Conc. (ng/mL)	Average Signal	%CV
0	117	8.2
0.024	546	0.4
0.098	1801	3.5
0.39	6948	3.6
1.6	27 887	5.4
6.3	103 243	2.9
25	265 562	5.0
100	420 068	0.9

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 based on 12 runs.

	Clusterin
Average LLOD (ng/mL)	0.0012
LLOD Range (ng/mL)	0.00098–0.0016

Precision

Rat urine-based controls with high and low levels of analytes were measured using a minimum of 2 replicates on 12 runs over 4 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 12 runs.

	Control	Runs	Average Conc. (ng/mL)	Average Intra-run %CV	Inter-run %CV
Clusterin	High	12	13	3.8	11.3
	Low	12	0.28	2.6	10.5

Dilution Linearity

To assess linearity, rat urine samples were diluted 2.5-fold, 5-fold, 10-fold, and 20-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.

% Recovery=measured/expected∗100

		Clusterin	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
Urine (N=3)	5	117	108–129
	10	107	104–113
	20	105	98–110

Spike Recovery

Rat urine samples were diluted 10-fold and spiked with calibrator at multiple levels throughout the range of the assay. The average percent recovery shown below was calculated from samples with values above the LLOD.

% Recovery=measured/expected∗100

	Clusterin		
Sample Type	Spike Conc. (ng/mL)	Average % Recovery	% Recovery Range
Urine (N=4)	50	86	85–86
	10	86	83–87
	1.0	82	79–85

Stability

Kit components were tested for freeze–thaw stability. Results (not shown) demonstrated that diluent and calibrator can go through 5 freeze–thaw cycles without significantly affecting assay performance.

Tested Samples

Urine samples were collected from normal Sprague-Dawley rats, diluted 10-fold, and tested with the Rat Clusterin assay. Median and range of concentrations are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	Clusterin
Urine	Median (ng/mL)	2.5
	Range (ng/mL)	<LLOD–88
	Number of Samples	41
	Samples above LLOD	39

Assay Components

Calibrator

Recombinant rat clusterin (residues 146-360) was expressed in *E. coli.*

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Clusterin	Goat Polyclonal	Goat Polyclonal

References

1. Araki S, et al. Clusterin proteins: stress-inducible polypeptides with proposed functions in multiple organ dysfunction. BJR Suppl. 2005;27:106-13.
2. Rached E, et al. Evaluation of Putative Biomarkers of Nephrotoxicity after Exposure to Ochratoxin A in vivo and in vitro. Toxicol Sci. 2008 Jun; 103(2):371-81.

Summary Protocol

MSD 96-well MULTI-ARRAY Rat Clusterin Kit

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

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Blocker A solution.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 29.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 10-fold in Diluent 29 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 29.
- ❑ Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1 : Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A Solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2 : Wash and Add Sample

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 50 µL/well of sample (standards or unknowns).
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3 : Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Read Plate

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

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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