MSD® MULTI-ARRAY Assay System

Human YKL-40 Kit

1-Plate Kit	K151NHD-1
5-Plate Kit	K151NHD-2
25-Plate Kit	K151NHD-4

MSD Inflammation Assays

Human YKL-40 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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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

YKL-40 (also known as human cartilage glycoprotein 39; HC-gp39) is a 40 kDa inflammatory glycoprotein involved in the activation of the innate immune system and extracellular matrix remodeling. YKL-40 is secreted by macrophages, neutrophils, chondrocytes, vascular smooth muscle, and hepatic stellate cells.[1] Elevated serum YKL-levels are associated with the presence and extent of coronary artery disease (CAD) and even higher YKL-40 levels have been documented in patients with myocardial infarction and both type 1 and type 2 diabetes.[2,3] Enhanced expression of YKL-40 is observed in macrophages and smooth muscle cells in atherosclerotic plaques. In endothelial dysfunction, elevated YKL-40 levels seem to be involved in cell migration, reorganization, and tissue remodeling in response to endothelial damage.[2,3] Therefore, YKL-40 may play a role in multiple pathogenic processes related to inflammation, extracellular tissue remodeling, fibrosis, and the metastatic and angiogenic invasiveness of many solid tumors.[2-4]

Principle of the Assay

MSD inflammation assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human YKL-40 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 a SECTOR® Imager 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.

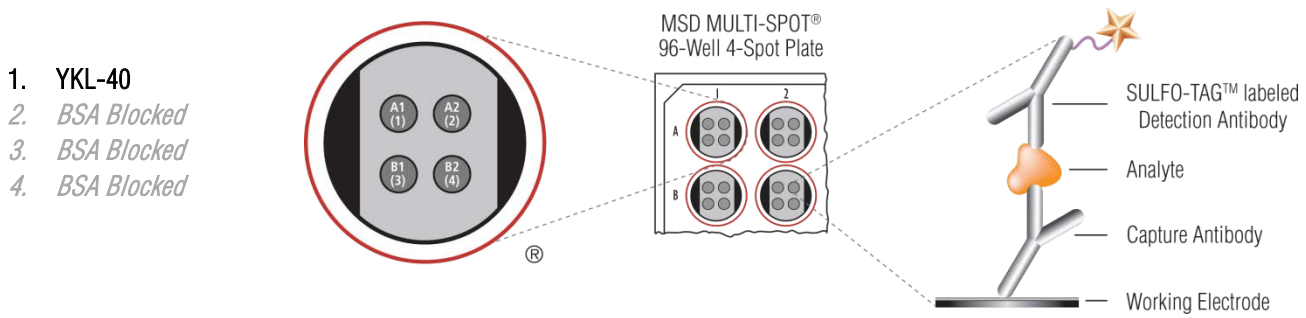


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151NHD-1	K151NHD-2	K151NHD-4
MULTI-SPOT 96-Well 4-Spot Human YKL-40 Plate N451NHA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu YKL-40 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human YKL-40 Calibrator (1 µg/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Diluent 100 R50AA-4 (50 mL), R50AA-2 (200 mL)	2–8°C	1 bottle (50 mL)	1 bottle (200 mL)	5 bottles (200 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.

[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 3 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 calibrator for the Human YKL-40 Kit 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. Prepare the standard solutions at room temperature.

Standard	YKL-40 Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	1 000 000	
STD-01	50 000	20
STD-02	12 500	4
STD-03	3125	4
STD-04	781	4
STD-05	195	4
STD-06	49	4
STD-07	12.2	4
STD-08	0	n/a

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

1) Prepare the highest standard (STD-01) by adding 25 µL of stock calibrator to 475 µL of Diluent 100. Mix well.
2) Prepare the next standard (STD-02) by transferring 100 µL of STD-01 to 300 µL of Diluent 100. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 100 as the blank.

Dilute Samples

Avoid multiple freeze–thaw cycles for serum and plasma samples. Dilute serum and plasma samples at least 50-fold in Diluent 100. Plasma prepared in heparin tubes commonly displays additional clotting following thawing of the sample. Remove clots and all solid material by centrifugation.

Prepare Detection Antibody Solution

MSD provides 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-hu YKL-40 Antibody
- ❑ 2940 µL of Diluent 3

Prepare Read Buffer

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

For 1 plate, combine:

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

Notes

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.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample:** Wash the plate 3 times with 300 µL/well of 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 (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 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 keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

   You may prepare diluted read buffer during incubation.

   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.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

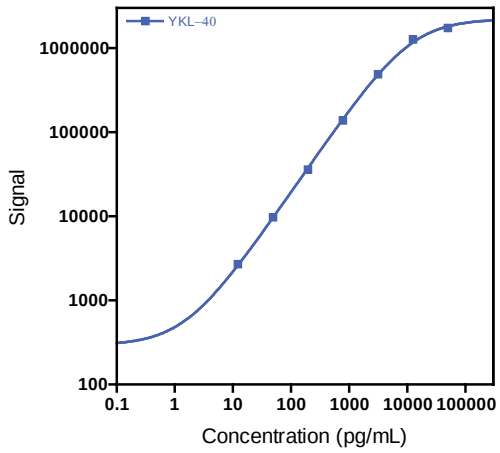
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the 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.



YKL-40		
Conc. (pg/mL)	Average Signal	%CV
0	292	6.0
12.2	2700	5.9
49	9766	2.7
195	35 997	2.6
781	138 070	2.8
3125	489 784	2.6
12 500	1 267 891	3.0
50 000	1 735 142	0.8

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).

	YKL-40
Average LLOD (pg/mL)	0.22

Assay Components

Calibrator

The assay calibrator uses recombinant human YKL-40 protein expressed in mouse myeloma cell line.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
YKL-40	Mouse Monoclonal	Rabbit Polyclonal

References

1. Roslind A, Johansen JS. YKL-40: a novel marker shared by chronic inflammation and oncogenic transformation. Methods Mol Biol. 2009;511:159-84.
2. Rathcke CN, Vestergaard H. YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. Inflamm Res. 2006 Jun;55(6):221-7.
3. Rathcke CN, Vestergaard H. YKL-40--an emerging biomarker in cardiovascular disease and diabetes. Cardiovasc Diabetol. 2009 Nov 23;8:61.
4. Johansen JS, et al. Serum YKL-40, a new prognostic biomarker in cancer patients? Cancer Epidemiol Biomarkers Prev. 2006 Feb;15(2):194-202.

Summary Protocol

Human YKL-40 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human YKL-40 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 100.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 50-fold in Diluent 100 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting the stock detection antibody 50-fold in Diluent 3.
- ❑ Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-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, controls, 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 2X Read Buffer T.
- ❑ Analyze plate on SECTOR Imager.

Plate Diagrams