MSD® MULTI-SPOT Assay System

Human A2M Kit

1-Plate Kit	K151JQD-1
5-Plate Kit	K151JQD-2
25-Plate Kit	K151JQD-4

MSD Biomarker Assays

Human A2M 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 Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com

Table of Contents

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

Alpha-2-macroglobulin (A2M) is the largest non-immunoglobulin protein present in plasma and a major representative of the alpha-macroglobulin family, which includes complementary proteins C3 and C4 as well as pregnancy zone protein (PZP). A2M can be locally synthesized by macrophages, fibroblasts, and adrenocortical cells,[1] but plasma A2M levels are mainly determined by liver synthesis. A2M is a 720 kDa protein composed of four identical 180 kDa subunits, each with 5 different reactive sites.[1] A2M functions as an antiproteinase by inhibiting all four classes of proteinases (including serine-, cysteine-, aspartic- and metalloproteinases).[2] It does this by using a unique trapping mechanism dependent on a 35 amino acid peptide stretch known as the bait region. This region contains specific cleavage sites for virtually all the different classes of proteinases, self and foreign. Proteinase cleavage at the bait region results in an immediate conformational change of A2M linked to cleavage of a reactive internal thioester bond. Cleavage of the thioester bond reveals reactive glutamyl and cysteinyl residues that can also covalently trap the proteinase. The conformational change in the protein also exposes the C-terminal receptor binding domain that allows the protein complex to bind to macrophage clearance receptors, mainly low-density lipoprotein related receptor (LRP/A2MR).[3] Due to its ability to inhibit plasma proteinases, A2M functions as an inhibitor of fibrinolysis and coagulation via plasmin/kallikrein and thrombin, respectively.[4]

In addition to trapping proteinases, A2M binds to many cytokines and growth factors including platelet-derived growth factor, basic fibroblast growth factor, TGF-β, insulin, and IL-1β.[1]

Plasma A2M levels are used as a diagnostic biomarker for kidney failure in nephrotic syndrome.[5] A2M also associates with amyloid-beta in the brain and is a late stage marker of Alzheimer's disease.[6]

Principle of the Assay

MSD biomarker assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human A2M 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.

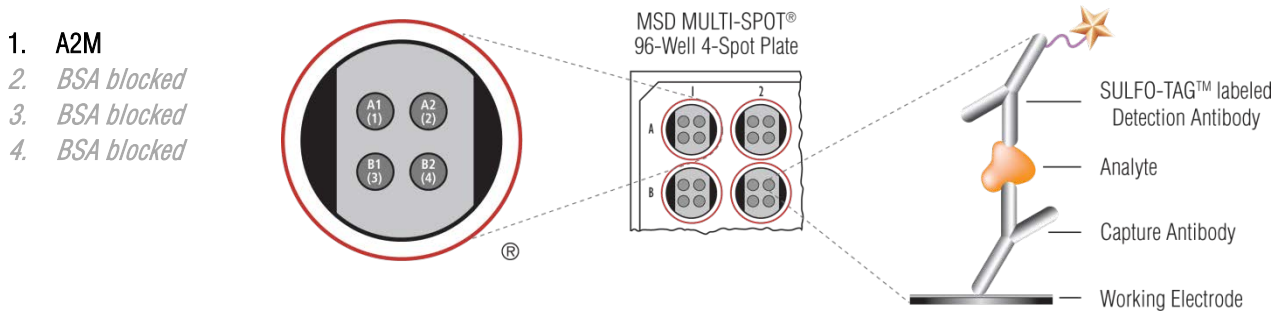


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151JQD-1	K151JQD-2	K151JQD-4
MULTI-SPOT 96-Well, 4-Spot Human A2M Plate N451JQA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu A2M Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human A2M Calibrator[2] (20X)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 100 R50AA-2 (200 mL)	2–8°C	1 bottle (200 mL)	2 bottles (200 mL)	10 bottles (200 mL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL ea)	5 bottles (25 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.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

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

[2] **Biohazard Statement:** This product was derived from human material and should be considered potentially infectious. Appropriate precautions should be used when handling this material.

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 A2M Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-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.

Standard	Human A2M (ng/mL)	Dilution Factor
Stock Calibrator	12 500	
STD-01	625	20
STD-02	208	3
STD-03	69.4	3
STD-04	23.1	3
STD-05	7.72	3
STD-06	2.57	3
STD-07	0.857	3
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 by adding 15 µL of stock calibrator to 285 µL of Diluent 100. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 200 µL of Diluent 100. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 100 as the blank.

Dilute Samples

For human serum and plasma samples, MSD recommends 4000-fold dilution in Diluent 100; however, you may need to adjust the dilution factor for the sample set under investigation.

Samples should be prepared in two dilution steps as follows:

1) Add 10 µL of sample to 390 µL of Diluent 100 (40-fold dilution)
2) Add 10 µL of the 40-fold diluted sample to 990 µL of Diluent 100 (100-fold dilution).

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 A2M Antibody
- ❑ 2940 µL of Diluent 3

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

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 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare diluted read buffer during incubation.

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

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 the SECTOR Imager. No incubation in read buffer is required before reading the plate.

   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.

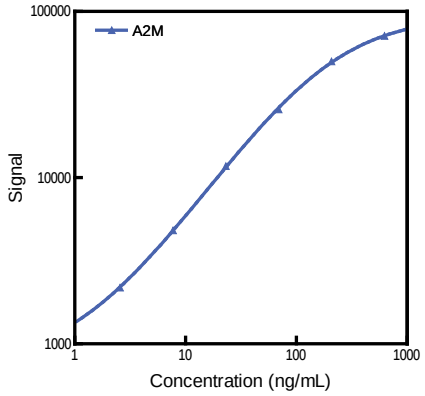
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

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



A2M		
Conc. (ng/mL)	Average Signal	%CV
0	844	3.7
0.857	1267	1.8
2.57	2181	3.3
7.72	4815	1.3
23.1	11 724	0.5
69.4	25 754	1.8
208	50 087	1.5
625	71 189	2.6

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 2 runs.

	A2M
LLOD Range (ng/mL)	0.908–0.958

Assay Components

Calibrator

The assay calibrator uses human plasma derived A2M protein.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
A2M	Goat Polyclonal	Goat Polyclonal

References

1. Borth W. Alpha 2-macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J. 1992 Dec;6(15):3345-53
2. Harpel PC. Studies on human plasma alpha 2-macroglobulin-enzyme interactions. Evidence for proteolytic modification of the subunit chain structure. J Exp Med. 1973 Sep 1;138(3):508-21.
3. Enghild JJ, et al. A conserved region in alpha-macroglobulins participates in binding to the mammalian alpha-macroglobulin receptor. Biochemistry. 1989 Feb 7;28(3):1406-12.
4. de Boer JP, et al. Alpha-2-macroglobulin functions as an inhibitor of fibrinolytic, clotting, and neutrophilic proteinases in sepsis: studies using a baboon model. Infect Immun. 1993 Dec;61(12):5035-43.
5. de Sain-van der Velden MG, et al. Plasma alpha 2 macroglobulin is increased in nephrotic patients as a result of increased synthesis alone. Kidney Int. 1998 Aug;54(2):530-5.
6. Kovacs DM. alpha2-macroglobulin in late-onset Alzheimer's disease Exp Gerontol. 2000 Jul;35(4):473-9.

Summary Protocol

Human A2M Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human A2M 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 3-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 4000-fold in Diluent 100 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 3.

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, 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 1 hour.

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 SECTOR Imager.

Plate Diagrams