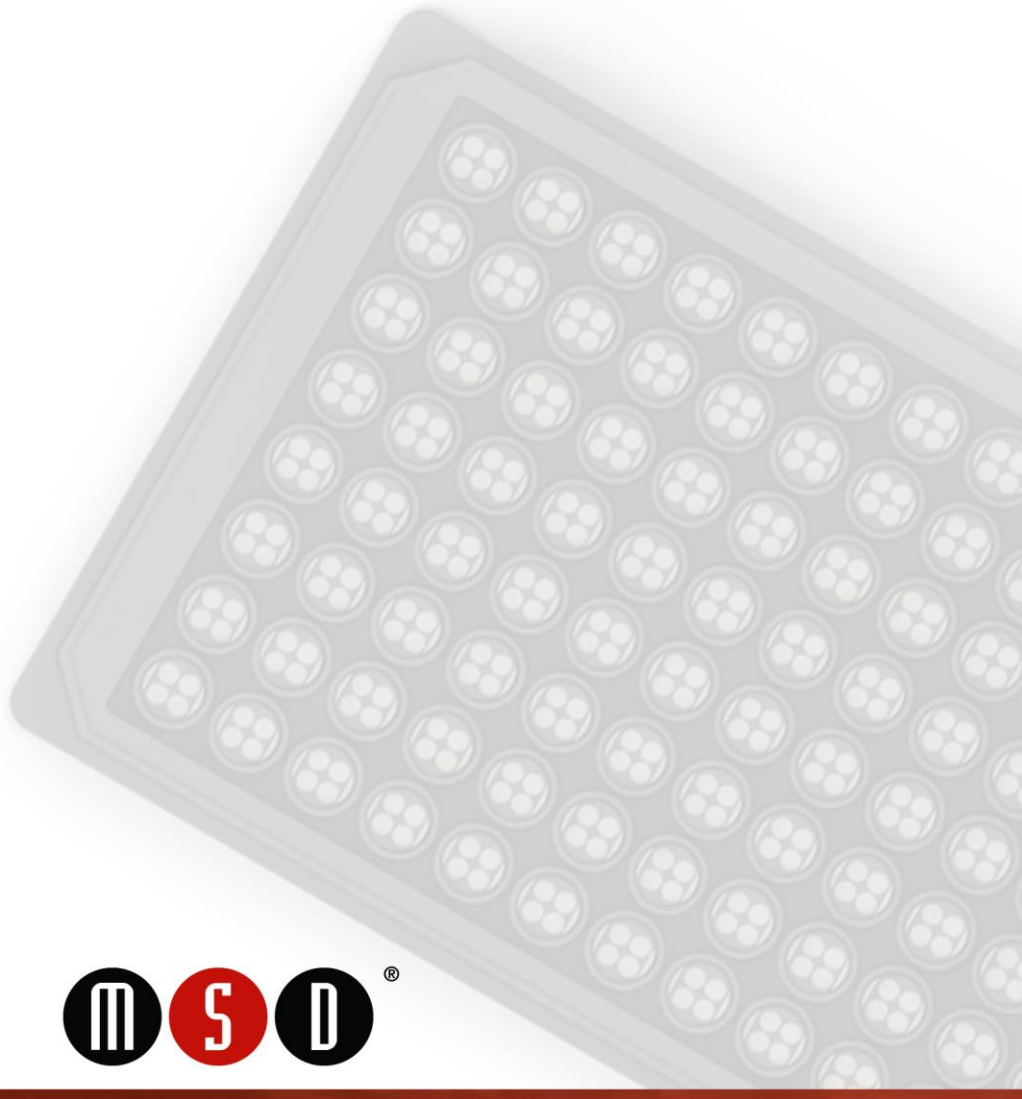
MSD® MULTI-ARRAY Assay System

Human Angiopoietin-1 Kit

1-Plate Kit	K151LPD-1
5-Plate Kit	K151LPD-2
25-Plate Kit	K151LPD-4



MSD Vascular Assays

Human Angiopoietin-1 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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Introduction

Angiopoietin-1 (Ang-1) plays a role in the modulation of blood vessel plasticity and contributes to vascular maintenance. Ang-1 enhances survival and migration of endothelial cells and induces neovascularization under both normal and pathogenic pro-angiogenic conditions. It is expressed in many adult human tissues, primarily by endothelial support cells, megakaryocytes, and platelets.[1,2] Despite their often opposing regulatory roles in angiogenesis, both Ang-1 and angiopoietin-2 (Ang-2) are ligands for the endothelial cell receptor tyrosine kinase, Tie-2.

Ang-1/Tie-2 signaling promotes angiogenesis during the development, remodeling, and repair of the vascular system. These interactions are complex and often mediated by the local cytokine and growth factor microenvironment.[2] Ang-1/Tie-2 signaling also plays a key role in neuronal cell proliferation and survival and in the maintenance of hematopoietic stem cells in non-proliferative states in the bone marrow. Elevated levels of Ang-1 have been observed in several human cancers and are correlated with tumor angiogenesis, growth, and progression.[3] Therefore, targeting angiopoietin/Tie-2 signaling pathways is a fertile strategy in the development of novel anti-tumor therapeutics.[3,4]

Principle of the Assay

MSD assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Human Angiopoietin-1 assay 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 intensity of emitted light to provide a quantitative measure of analytes in the sample.

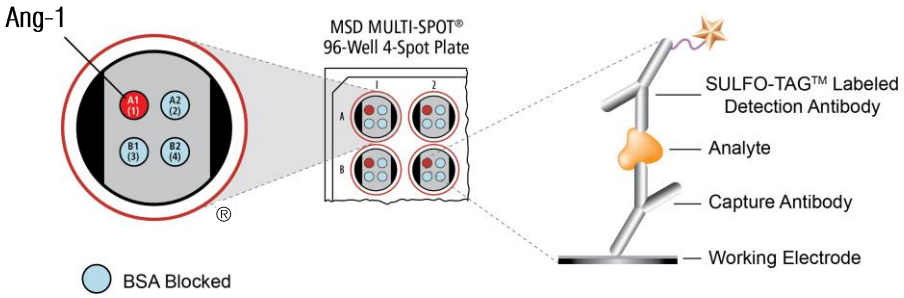


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K151LPD-1	K151LPD-2	K151LPD-4
MULTI-SPOT® 96-Well 4-Spot Human Angiopoietin-1 Plate N451LPA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Ang-1 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Ang-1 Calibrator (2 µg/mL)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 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 7 R54BB-3 (50 mL)	≤-10°C	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (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)

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing or MSD Wash Buffer (Catalog No. R61AA-1)
- ❑ 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 (rotary) capable of shaking at 500-1,000 rpm
- ❑ Deionized water
- ❑ Vortex

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 product-specific safety information is available in the Safety Data Sheet (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

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

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific certificate of analysis (COA).
- Assay incubation steps should be performed between 20-26°C to achieve the most consistent signals between runs.
- Bring frozen diluent (if applicable) to room temperature in a 24°C water bath. Thaw other reagents on wet ice and use as directed without delay.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding Read Buffer T may interfere with signal detection.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette to the bottom corner.
- Shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove the plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors (see sector map in instrument and software manuals) to avoid contaminating unused wells. Remove all seals before reading. Partially used plates may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

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

> **Important:** Upon first thaw, separate Diluent 3 and Diluent 7 into suitably-sized aliquots before refreezing. These diluents can go through 3 freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Calibrator Standards

MSD recommends an 8-point standard curve with 4-fold serial dilution steps and a zero calibrator. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	Ang-1 Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	2,000,000	
STD-01	100,000	20
STD-02	25,000	4
STD-03	6,250	4
STD-04	1,563	4
STD-05	391	4
STD-06	98	4
STD-07	24	4
STD-08	0	n/a

To prepare an 8-point standard curve for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of the calibrator stock to 285 µL of Diluent 7. Mix well.
2) Prepare the next standard by transferring 60 µL of the highest standard to 180 µL of Diluent 7. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 7 as the 8th standard (i.e. zero calibrator).

Standards should be prepared at room temperature no more than 20 minutes before use.

Dilute Samples

For serum and plasma samples, MSD recommends a 2-fold dilution in Diluent 7; however you may adjust dilution factors for the sample set under investigation.

Prepare Detection Antibody Solution

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

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Ang-1 Antibody
- ❑ 2.94 mL of Diluent 3

Prepare Wash Buffer

MSD provides Wash Buffer as a 20X stock solution. The working solution is 1X. PBS + 0.05% Tween-20 can be used instead.

For one plate, combine:

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

Prepare Read Buffer

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

For one plate, combine:

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

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: **Add Blocker A**

- ❑ Add 150 µL of Blocker A Solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (500-1,000 rpm) for 1 hour.

STEP 2: **Add Sample or Calibrator**

- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 50 µL of Calibrator or diluted sample to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (500-1,000 rpm) for 2 hours.

STEP 3: **Wash and Add Detection Antibody Solution**

- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (500-1,000 rpm) for 2 hours.

STEP 4: **Wash and Read**

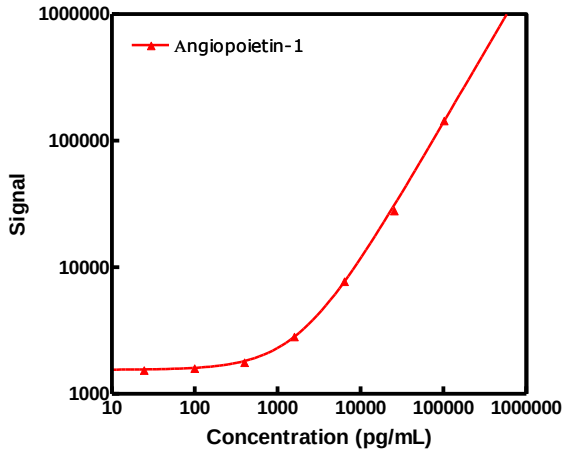
- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer T is not required before reading the plate.

Analysis of Results

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) which allows accurate quantification without the need for dilution in many cases. 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 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.



	Angiopoietin-1	
Conc. (pg/mL)	Average Signal	%CV
0	1,505	1.1
24	1,550	6.6
98	1,609	2.9
391	1,788	4.4
1563	2,860	0.8
6250	7,821	2.7
25 000	28,265	2.0
100 000	145,473	1.7

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the blank (zero calibrator).

	Angiopoietin-1
LLOD (pg/mL)	26

Assay Components

Calibrator

The assay calibrator uses recombinant human Ang-1 protein, residues 20-498, expressed in NSO derived murine myeloma cell line.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Ang-1	Mouse Monoclonal	Goat Polyclonal

References

1. Thomas M, Augustin HG. The role of the Angiopoietins in vascular morphogenesis. Angiogenesis. 2009 12(2):125-37.
2. Fiedler U, Augustin HG. Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol. 2006 Dec;27(12):552-8.
3. Wu X, Liu N. The role of Ang/Tie signaling in lymphangiogenesis. Lymphology. 2010 Jun;43(2):59-72.
4. Peters KG, Kontos CD, Lin PC, et al. Functional significance of Tie2 signaling in the adult vasculature. Recent Prog Horm Res. 2004 59:51-71.

Summary Protocol

MSD 96-well MULTI-ARRAY® Human Angiopoietin-1 Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Human Angiopoietin-1 assay.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature, and thaw the calibrator on ice.
- ❑ Prepare Blocker A solution.
- ❑ Prepare 8 standard solutions using the supplied calibrator as described in the "Prepare Standards" section.
- ❑ Dilute samples 2-fold in Diluent 7 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting 50X detection antibody 50-fold in Diluent 3.
- ❑ Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.
- ❑ If using MSD Wash Buffer, dilute stock 20X MSD Wash Buffer 20-fold with deionized water to prepare a 1X working solution.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A Solution.
- ❑ Incubate at room temperature with shaking (500–1,000 rpm) for 1 hour.

STEP 2: Wash and Add Sample or Calibrator

- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 50 µL/well of calibrator or diluted sample.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with shaking (500–1,000 rpm) for 2 hours.

STEP 4: Wash and Read Plate

- ❑ Wash the plate three times with at least 150 µL/well of PBS-T or 1X MSD Wash Buffer.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze the plate on an MSD instrument.

Plate Diagrams