MSD MULTI-ARRAY® Assay System

Human Angiopoietin-2 Kit

1-Plate Kit	K151KCD-1
5-Plate Kit	K151KCD-2
25-Plate Kit	K151KCD-4

MSD® Vascular Assays

Human Angiopoietin-2 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

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Introduction

Angiopoietin-2 (Ang-2) is a 66 kDa protein that plays a crucial role in maturation and maintenance of the vascular and lymphatic systems. Ang-2 expression occurs primarily in Weibel-Palade bodies on endothelial cells and at sites of active vascular remodeling, such as the placenta, ovaries, and uterus.[1] Despite their often opposing regulatory roles in angiogenesis, both Angiopoietin-1 (Ang-1) and Ang-2 are ligands for the endothelial cell receptor tyrosine kinase, Tie-2. Ang-2/Tie-2 interactions are complex and often mediated by the local cytokine and growth factor microenvironment.[2]

Ang-2 modulates angiogenesis in a cooperative manner with vascular endothelial growth factor (VEGF), facilitating endothelial cell migration, proliferation, and vascular sprouting. However, in the absence of VEGF, Ang-2 induces endothelial cell death and vascular regression, and when bound to Tie-2, counteracts the blood vessel maturation and stability mediated by Ang-1.[2-4] Ang-2 and its effect on angiogenesis have been implicated in human cancers of the lung, colon, liver, and gut and may be correlated with tumor progression and poor outcomes. 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 toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Human Angiopoietin-2 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 a SECTOR® Imager 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.

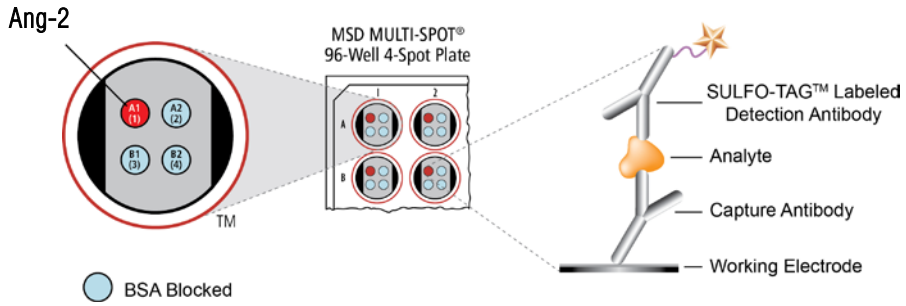


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit K151KCD-1	K151KCD-2	K151KCD-4
MULTI-SPOT 96-Well 4-Spot Human Angiopoietin-2 Plate N451KCA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Ang-2 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Ang-2 Calibrator (0.2 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)

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

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 and Diluent 7 into aliquots appropriate to the size of your assay needs. 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 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-2 Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	200 000	
STD-01	10 000	20
STD-02	2500	4
STD-03	625	4
STD-04	156	4
STD-05	39	4
STD-06	9.8	4
STD-07	2.4	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 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Ang-2 Antibody
- ❑ 2.94 mL 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 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.

Assay Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL 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.

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

2. **Wash and Add Sample or Calibrator:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 50 µL of calibrator or diluted sample 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 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.

   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 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

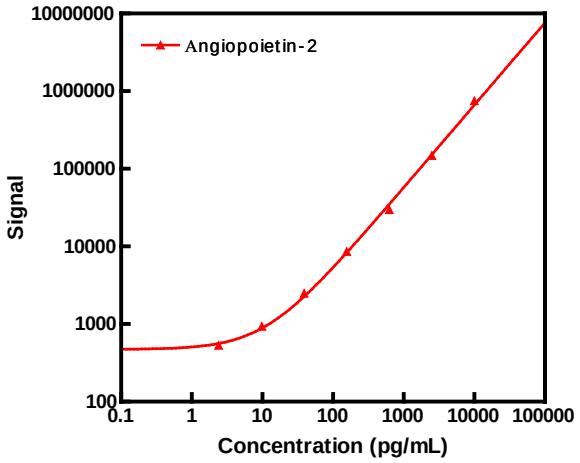
Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

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-2	
Conc. (pg/mL)	Average Signal	%CV
0	382	5.2
2.4	534	9.0
9.8	937	2.6
39	2482	2.0
156	8583	0.1
625	29 756	7.4
2500	148 076	0.9
10 000	754 463	3.3

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-2
LLOD (pg/mL)	1.2

Assay Components

Calibrator

The assay calibrator uses recombinant human Ang-2 protein, residues 19-496, expressed in NSO derived murine myeloma cell line.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Ang-2	Mouse Monoclonal	Mouse Monoclonal

References

1. Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. The Tie-2 ligand Angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood. 2004 103: 4150-6.
2. Yuan HT, Khankin EV, Karumanchi SA, et al. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol. 2009 29:2011-22.
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-2 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Angiopoietin-2 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.

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 or Calibrator

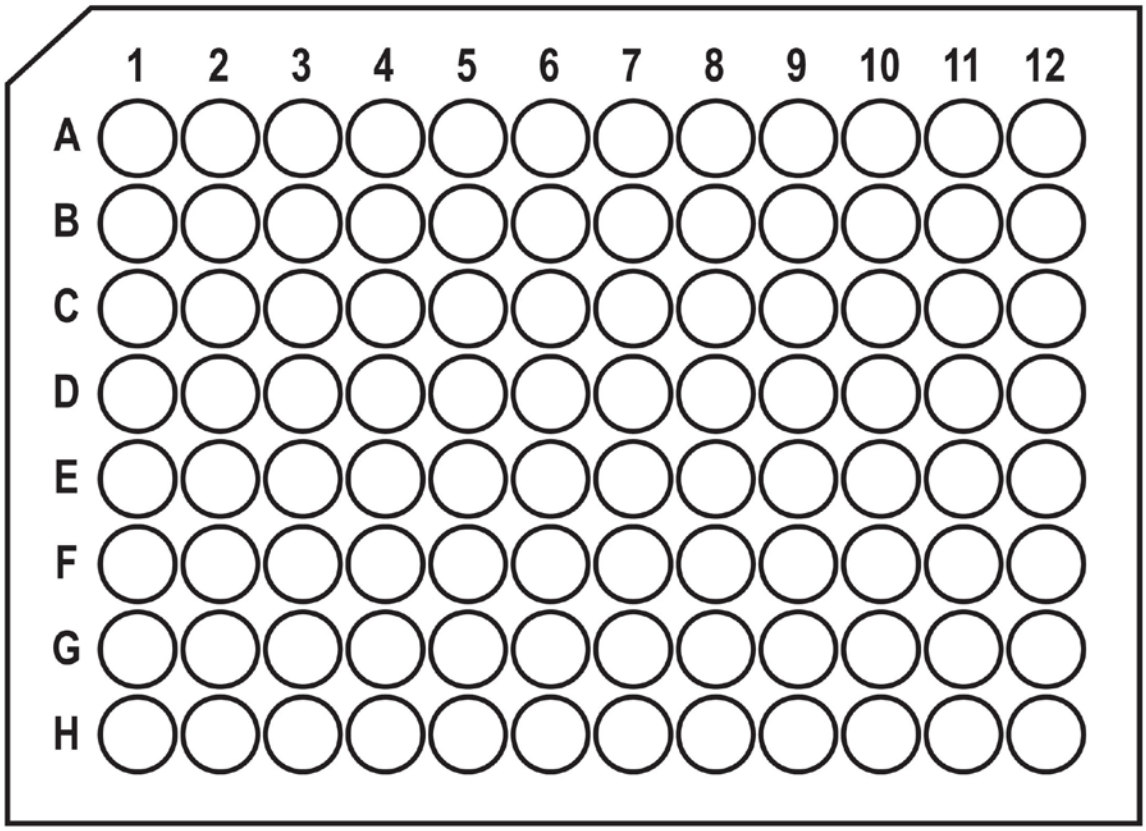
Wash plate 3 times with 300 µL/well of PBS-T.
Add 50 µL/well of calibrator or diluted sample.
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

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.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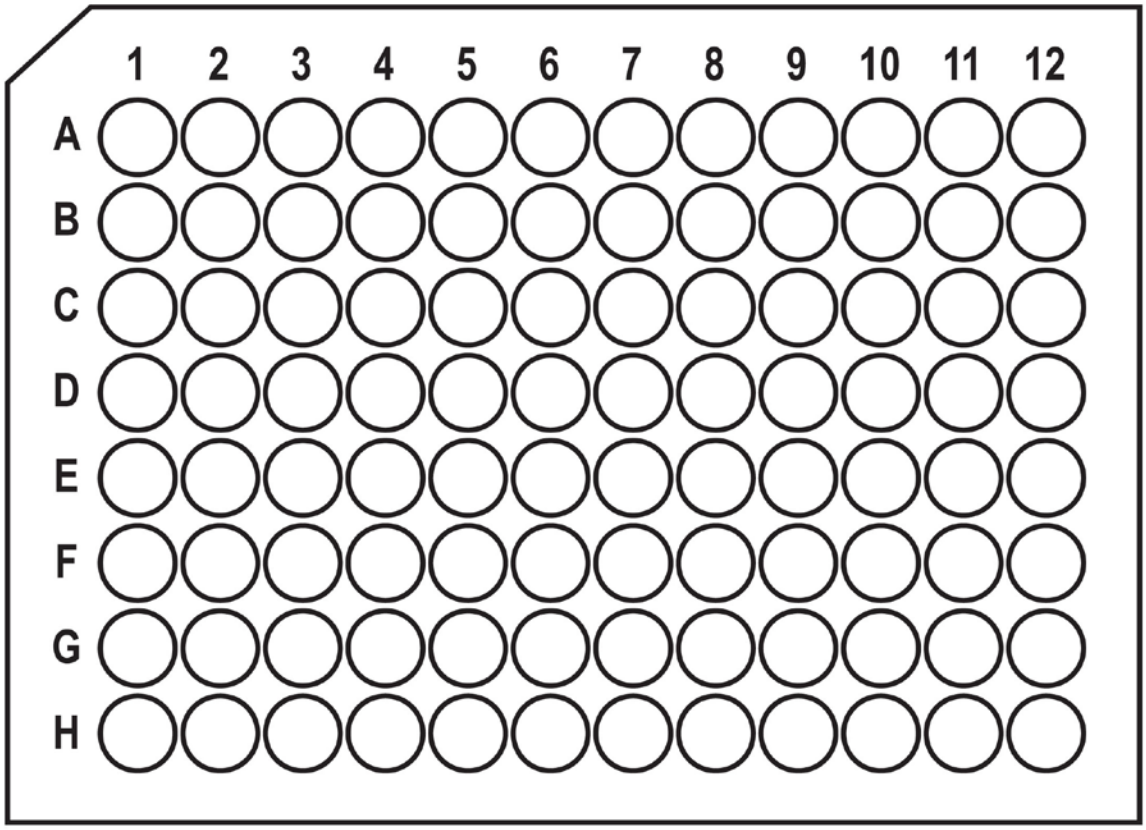
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