Meso Scale Discovery

MULTI-ARRAY™ 96-well Anti-Species Antibody Coated Plates

Table of Contents

table of contents

Contact Information

contact information

MSD Customer Service
Phone: 240-631-2522
Fax: 301-990-2776
Email: sales@mesoscale.com

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Introduction

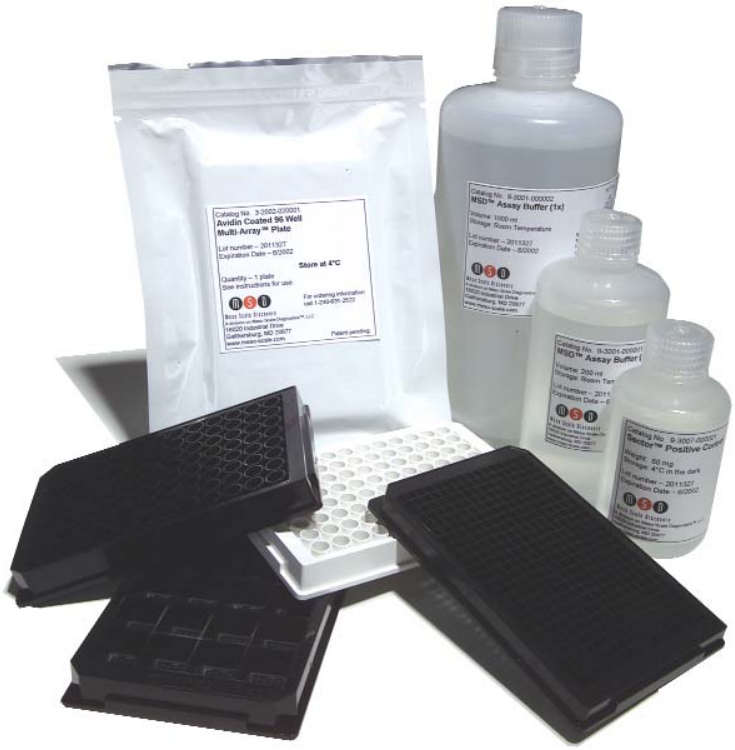
introduction

MSD's anti-species antibody coated MULTI-ARRAY plates offer exceptional convenience and flexibility for the development of assays on the MSD platform. Anti-species antibody coated MULTI-ARRAY plates offer flexibility in sandwich assay development as the solution formulation of the capture antibody is not limiting, and may contain serum or carrier protein.

The MSD platform provides a faster and less labor-intensive alternative to traditional ELISAs for performing sandwich immunoassays. Anti-species antibody coated 96-well MULTI-ARRAY plates are ideal for the development of singleplex sandwich immunoassays. These assays use a capture antibody (polyclonal from crude sera) that is bound to the anti-species antibody coated surface of a MULTI-ARRAY plate and a detection antibody that is labeled with MSD SULFO-TAG™ reagent, an electrochemiluminescent label. In addition, these plates can be used as a tool for identifying antibodies and conditions that can be transferred to array-based multiplex sandwich immunoassays on MSD's MULTI-SPOT® plates.

This technical note provides detailed instructions for the use of anti-species antibody coated MULTI-ARRAY plates as well as instructions on the use of these plates in conjunction with MSD SULFO-TAG labeled detection antibodies for performing sandwich immunoassays on MSD's SECTOR PR™ and SECTOR™ Imager instruments. It provides summary and detailed protocol information for experimental design, suggested initial assay development experiments, and a detailed list of important facts to remember when working with MULTI-ARRAY plates and SULFO-TAG labeled detection antibodies.

MSD also supplies MULTI-ARRAY plates that are uncoated to allow users to customize the plate coating for their specific application of interest, or plates that are pre-coated with avidin or streptavidin for the immobilization of biotinylated material. Detailed protocols for the use of these uncoated or pre-coated plates are described in separate technical notes, available at www.mesoscale.com. Contact MSD Technical Support for additional information regarding applications for anti-species antibody coated MULTI-ARRAY plates.

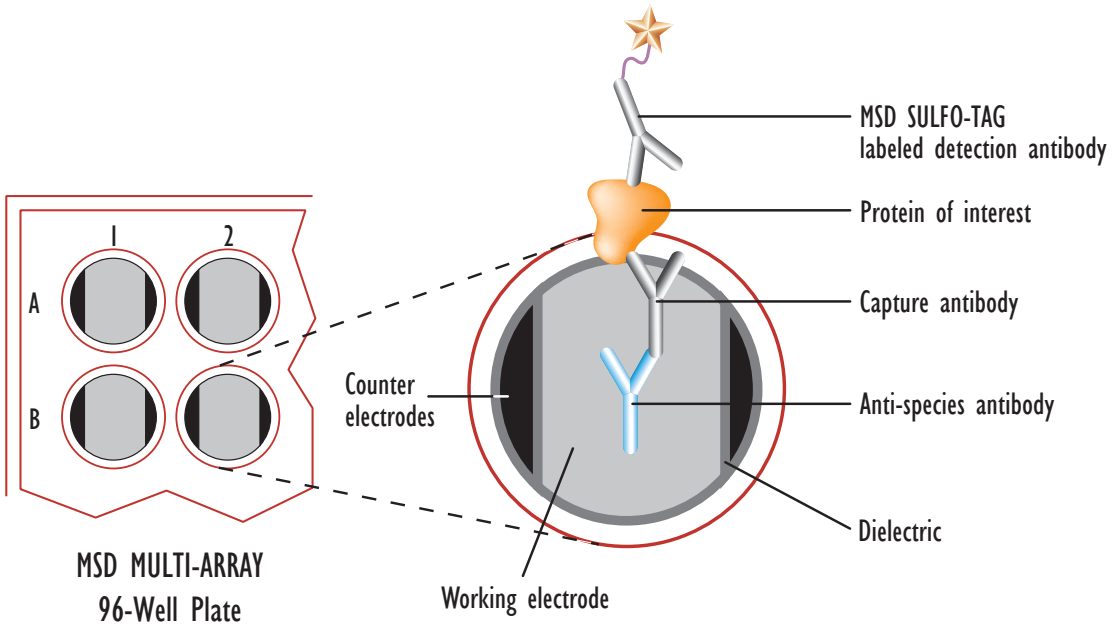


Principle of the Assay

principle of the assay

MSD assay technology provides a rapid and convenient method for measuring one or more protein targets within a single small-volume sample. Our anti-species MULTI-ARRAY 96-well plates supply a platform for the development of sandwich immunoassays (Figure 1). MSD provides a plate that is coated with an anti-species antibody that enables the user to immobilize a primary capture antibody against a protein of interest (target). The user then adds the sample and a solution containing the labeled detection antibody (an anti-target antibody labeled with an electrochemiluminescent compound, MSD SULFO-TAG label) over the course of one or more incubation periods. The target present in the sample binds to the capture antibody immobilized on the working electrode surface by the anti-species antibody; recruitment of the labeled detection antibody by the bound target completes the sandwich. The user adds an MSD Read Buffer that provides the appropriate chemical environment for ECL and loads the plate into an MSD SECTOR™ instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the label bound to the electrode surface to emit light. The instrument measures intensity of the emitted light to afford a quantitative measure of the amount of the protein of interest that is present in the sample.

Figure 1: *Sandwich immunoassay on MSD MULTI-ARRAY Plate*



III Protocol at a Glance

protocol at a glance

The following protocol describes the most conservative approach to developing highly sensitive sandwich immunoassays using anti-species coated MULTI-ARRAY plates.

Most optimized protocols require only 1-3 hours to complete. During initial development, MSD recommends using a conservative protocol that requires between 4 hours and overnight to complete, depending on the kinetics of each binding event. Once desirable results are achieved, the protocol can be streamlined to combine multiple incubations, eliminate wash steps, and shorten incubation times with only a slight loss of sensitivity in most cases. The following is a general starting protocol.

1. Add 150 µL blocking solution, incubate 1 hour, wash.
2. Add 25 µL capture antibody, incubate 1 hour, wash.
3. Add 25 µL samples, incubate 1 hour to overnight, wash.
4. Add 25 µL MSD SULFO-TAG labeled detection antibody, incubate 1-4 hours, wash.
5. Add 150 µL Read Buffer and analyze plate.

See Section 7, Detailed Assay Protocol, for additional information.

IV Facts to Remember

facts to remember

1. Capture Antibodies
   - Must be produced in a host species recognized by the anti-species coated electrode.
   - Can be in any form and may contain serum or carrier protein.
2. Plates
   a) Standard
      - Anti-species coated electrode surface is capable of binding 1 picomole of capture antibody from solution.

   b) High Bind
      - Anti-species coated electrode surface is capable of binding 5 picomoles of capture antibody from solution.
3. Detection Antibodies
   - Must be produced in a different host species than the capture antibody.
   - Must be free from glycerol, carrier protein, and amine-containing molecules such as azide, Tris, and glycine, that interfere with the process of labeling the antibody with MSD SULFO-TAG NHS Ester.
   - If a purified, carrier-free antibody cannot be obtained for labeling purposes, an MSD SULFO-TAG labeled anti-species secondary antibody may be used (must be produced in a host species other than the capture antibody).

V Samples

samples

A wide variety of samples can be used in the assay, including serum, plasma, whole blood, cell lysates, cells, conditioned cell culture medium, and more. Separate application notes are available that offer guidance on the manipulation of different matrices. Please contact MSD Customer Support for more information.

VI Assay Development Experiments

suggested initial experiments

The experiments outlined below provide a guide to initial sandwich assay development. The three assay variables that will be addressed are as follows: capture antibody concentration, detection antibody concentration, and incubation format. The results of these experiments will aid in determining the most suitable capture and detection antibodies for use in a specific sandwich immunoassay, as well as the most suitable incubation format. Further experimentation based upon these results may be required in order to find the best assay conditions. This experimental outline is a general guide, with incubation times, solution references, and other specific details addressed Section 7, Plate Detailed Assay Protocol.

Variable	Range
Capture antibody concentration	80, 40, 20, 0 nM *if using polyclonal antisera: 1:4000, 1:1000, 1:250, 0*
Detection antibody concentration	30, 10, 3, 1 nM
Incubation format	Stepwise or simultaneous

Experimental outline:

Please refer to Section 7 for detailed protocol information. A suggested Plate Layout for use with both formats is illustrated in Figure 2 below. Both plates should be ready for Read Buffer addition and analysis at the same time if concurrently performing both assay formats.

Stepwise Incubation format: (1 plate)

1. Add blocking solution and incubate.
2. Wash.
3. Add capture antibody and incubate.
4. Wash.
5. Add sample and incubate.
6. Wash.
7. Add detection antibody and incubate.
8. Wash.
9. Add Read Buffer and Analyze plate.

Simultaneous Incubation format: (1 plate)

1. Add blocking solution and incubate.
2. Wash.
3. Add capture antibody and incubate.
4. Wash.
5. Add sample *and* detection antibody, and incubate (for 2X the single incubation time used in the Stepwise format).
6. Wash.
7. Add Read Buffer and Analyze plate.

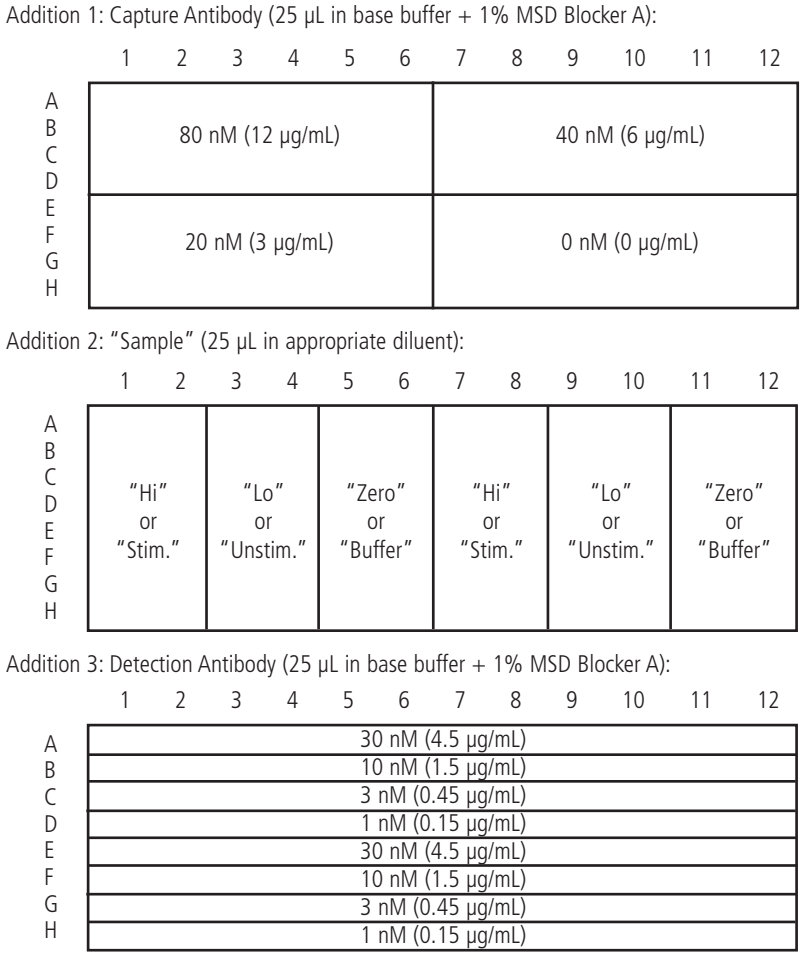


Figure 2: *Suggested plate layouts for assay development experiments.*

If increased assay throughput is desired, the final wash step prior to Read Buffer addition can be omitted with the use of a specialized Read Buffer. Many assays perform similarly in an "unwashed" format using an appropriate volume of concentrated MSD Read Buffer P to produce a final volume of 150 µL and a 1X concentration of Read Buffer in the well. Consult the MSD Read Buffer Application Note for more information.

VII Detailed Assay Protocol

detailed assay protocol

Label detection antibody:

This protocol does not describe labeling the detection antibody. This is typically performed once and adequate reagent is produced for many assays. It can be completed in advance or during the incubation periods in the protocol below using the method outlined in the MSD SULFO-TAG NHS Ester Application Note.

Prepare base buffer:

MSD recommends the use of a single common buffer throughout the assay to prepare all diluents and to wash the plates (e.g. PBS, Tris, HEPES). This will be referred to as "base buffer."

a) Prepare 250 mL per plate - more may be required if using an automatic plate washer.

Prepare blocking solution:

The MSD Blocker A Kit contains the materials to prepare a 3% blocker solution in PBS. This kit can be used as provided or the dry MSD Blocker A can be dissolved in a different buffer if desired:

a) In a 50 mL tube combine (per plate):

- 600 mg MSD Blocker A (30 mg/mL or 3%)
- 20 mL 1X base buffer

Prepare antibody dilution buffer:

MSD Antibody Diluent contains a blend of stabilizers including MSD Blocker A in PBS. This can be used as-is or MSD Blocker A can be added to a different buffer to make an alternative antibody diluent if desired:

a) Prepare a solution containing 10 mg/mL MSD Blocker A in base buffer. This can be done by diluting blocking solution from 30 mg/mL to 10 mg/mL.

b) In 15 mL tubes combine (do this twice per plate, you will need 3 mL for each antibody):

- 1.0 mL blocking solution
- 2.0 mL base buffer

Begin with an MSD MULTI-ARRAY Anti-Species Coated 96-well plate. No pretreatment is necessary.

Notes:

Experimental set up, labeling the antibody, and clean up can be completed in 2-3 hours. Read the MSD SULFO-TAG NHS Ester Application note thoroughly prior to beginning the procedure.

MSD MULTI-ARRAY plates are compatible with most buffers and cell culture media. A wide variety of solutions have been tested including most preservatives and reducing agents.

A larger amount of base buffer may be prepared at once and stored at room temperature for later use.

If working with phosphoproteins, MSD recommends avoiding the PBS-based buffer supplied with the MSD Blocker A Kit and MSD Antibody Diluent. Phosphate may interfere with some phosphospecific antibodies. Use of a Tris or HEPES based buffer is suggested.

Solutions containing MSD Blocker A should be kept at 4ºC and discarded after 14 days.

STEP 1

Add 150 µL/well of blocking solution. Do not touch the pre-coated electrode surface with the pipette tip.

Incubate at room temperature for 1-5 hours (or at 4ºC for 1-7 days for greater flexibility). Prepare capture antibody during this time.

Prepare capture antibody:

a) In a 15 mL tube combine (per plate):

- 3 mL antibody dilution buffer
- capture antibody

(See Section 6: Assay Development Experiments for suggested concentration to test.)

Wash plates four times with base buffer.

Notes:

When using multiple types of plate coatings, save the plate packaging or copy the serial numbers of each plate with the corresponding coating condition. Data files will carry the plate serial number but no information about the type of coating.

STEP 2

Add 25 µL/well of capture antibody.

Incubate at room temperature, shaking, until the binding equilibrium is achieved. This usually requires 1 hour. The exact time necessary will vary by application and needs to be determined experimentally. Prepare samples during this time.

Prepare samples:

a) Samples, including cell lysates, serum, etc., may be used neat or after dilution.
b) MULTI-ARRAY plates are compatible with most sample matrices. Avoid reagents that will denature the capture antibody (i.e. SDS should be 0.1% or less in the sample applied to the well).
c) Depending on the stability of the target in the matrix, additional protease or phosphatase inhibitors may be required in the diluent or matrix.
d) If working with purified protein, a few nanograms per well will generally provide a strong assay signal. Purified recombinant proteins may exhibit differences in both signal and background as compared to native proteins in cell lysate samples.
e) Keep diluted samples on ice until use.

Wash plates four times with base buffer.

Shaking a 96-well MSD MULTI-ARRAY or MULTI-SPOT® plate accelerates binding at the working electrode(s).

The sensitivity of MSD immunoassays rivals that of ELISAs and Western blots. The amount of sample required for a given assay will depend on the abundance of the analyte in the matrix and the affinities of the antibodies used.

Samples should not be serially diluted in the MSD plate.

STEP 3

Dispense 25 µL/well of diluted samples.

Incubate at room temperature, shaking, until the binding equilibrium is achieved. This usually requires 1-4 hours. The exact time necessary will vary by application and needs to be determined experimentally. Prepare detection antibody during this time.

Prepare detection antibody:

a) In a 15 mL tube combine (per plate):

- 3 mL antibody dilution buffer
- SULFO-TAG labeled detection antibody

(See Section 6 for suggested concentrations to test.)

Wash plates four times with base buffer.

If an indirect reporting method is used, an additional 1 hour incubation may be introduced in which 25µL of a 5nM secondary anti-species antibody solution follows the incubation with the unlabeled detection antibody. Ultimately, the protocol should evolve to a single step using both reagents at a 1:1 molar ratio.

STEP 4

Add 25 µL/well of detection antibody.

Incubate at room temperature, shaking, until the binding equilibrium is achieved. This usually requires between 1-2 hours. The exact time necessary will vary by application and needs to be determined experimentally. Prepare Read Buffer during this time.

Notes:

Extra diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

Dilute Read Buffer:

a) In a 50 mL tube combine (per plate):

- 15 mL deionized water
- 5 mL 4X MSD Read Buffer T

Wash plates four times with base buffer.

STEP 5

Carefully add 150 µL of diluted Read Buffer T, avoiding any bubbles.

The introduction of bubbles during Read Buffer addition to the wells will interfere with reliable imaging of the plate.

Analyze with SECTOR Imager or SECTOR PR™ 400 instrument:

1. Load plate into a stacker or place on single plate adapter.
2. Double click on DISCOVERY WORKBENCH® icon on computer desktop (if not already open).
3. Click the instrument icon in upper left corner of screen (if not already open to plate reading screen).
4. From the pull down menu select "Read From Barcode."
5. If only reading one plate check "Return Plate to Input Stack" (Imagers only).
6. Check the box and enter number of plates to be read.
7. Click the "Run" button.
8. Check the box to export default data file.
9. If desired, make selections to export a custom data file.
10. Browse and select the location to export data files.
11. Click OK to initiate the run.
12. Data will be automatically saved in the software database and text versions of the requested data files exported to the folder designated.

Plates can be imaged immediately following the addition of Read Buffer. Most MSD Immunoassays tolerate incubation in Read Buffer, however, each unique assay should be tested for stability in Read Buffer before being left to sit for extended periods.

Regardless of selections made for data file export, an all-inclusive indelible copy of the data and associated instrument information will be saved in the internal database. From this database additional copies of the data file may be exported in any layout at any time. Consult the instrument user manual.

NOTES:

VIII Referenced MSD Materials

referenced MSD materials

Items	Storage
MULTI-ARRAY 96 Anti-Species Antibody Coated Plates	4ºC
MSD Read Buffer T (4X) with surfactant [1]	RT
MSD Blocker A Kit, 1L	4ºC
50 g MSD Blocker A	
200 mL MSD Blocker A Buffer (5X PBS with preservatives)	
MSD SULFO-TAG NHS Ester	-20ºC

Other Application Notes:

MSD SULFO-TAG NHS Ester
MSD Blocker A
MSD Read Buffers

Other Technical Notes:

MSD MULTI-SPOT Phosphoprotein Assays
MULTI-ARRAY 96-well Plates
MULTI-ARRAY 96-well Avidin & Streptavidin Coated Plates

IX MSD Reagent Information

MSD reagent information

MSD Read Buffer T with surfactant:

A proprietary Tris buffered tripropylamine solution containing Triton X-100

MSD SULFO-TAG NHS Ester:

Ruthenium-tris-bipyridine N-hydroxysuccinimide ester

MSD Blocker A:

A mixture of blocking agents including bovine serum albumin

MSD Blocker A Buffer:

5X phosphate buffered saline (PBS) with preservatives

[1] *Additional Read Buffers are available for specialized use. Consult the MSD Read Buffer Application Note.*

X Multiplex Assays

multiplex assays

Once suitable antibodies have been identified for individual assays, they may be combined in multiplex assays. MSD invites customers to identify or send antibodies for immobilization on MULTI-SPOT plates, however, antibodies must be free of gelatin to be included in multiplex assays. If useful antibodies have been identified using indirect means, MSD will acquire suitable formulations of the commercially available antibodies and immobilize them on MULTI-SPOT electrodes or label them as needed. MSD can also receive your proprietary antibodies that can be immobilized on MULTI-SPOT plates or labeled as appropriate and returned for your use only. Moreover, MSD can prepare multiplex assays that utilize combinations of commercially available and proprietary antibodies. Contact MSD Customer Service for more information about this program.

XI Support

support

MSD Customer Support is available 9 AM - 5 PM Monday through Friday, excluding holidays.

Ordering Information

ordering information

MSD Customer Service
Phone: 240-631-2522
Fax: 301-990-2776
Email: sales@mesoscale.com

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

The products referenced in this technical note are for research use only, and are not for use in diagnostic or therapeutic procedures.