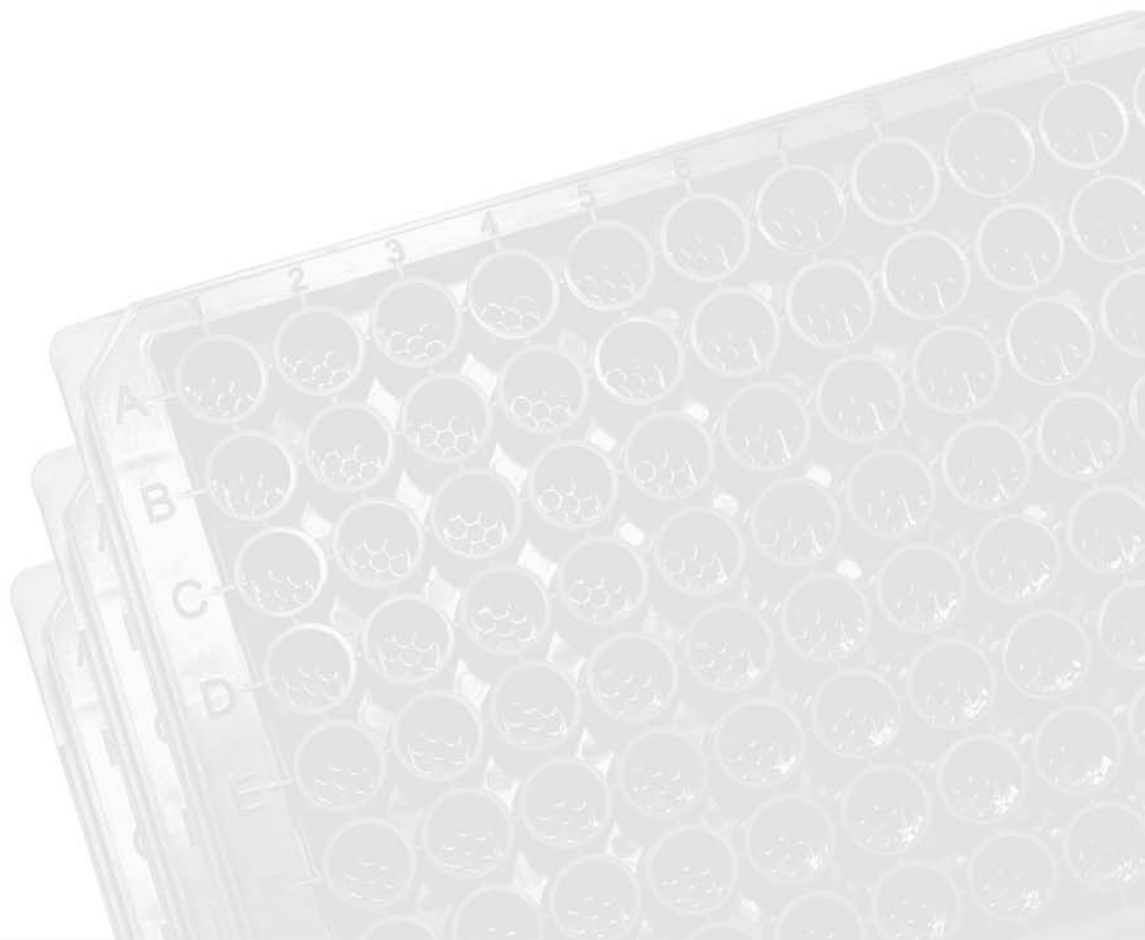


Meso Scale Discovery

MULTI-ARRAY™ 96-well Plates



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Introduction

introduction



MSD's uncoated MULTI-ARRAY plates offer exceptional convenience and flexibility for the development of assays on the MSD platform. MULTI-ARRAY plates can be used in many diverse applications ranging from the measurement of the abundance of cellular phosphoproteins, cytokines and other biomarkers to measuring the affinity of protein-protein interactions. The uncoated plates allow users to customize the plate coating for their specific application of interest.

The MSD platform provides a faster and less labor-intensive alternative to traditional ELISAs for performing sandwich immunoassays. Uncoated 96-well MULTI-ARRAY plates are ideal for the development of singleplex sandwich immunoassays. These assays use a capture antibody that is non-specifically adsorbed on the carbon 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 on the immobilization of capture antibodies on the carbon surface of uncoated 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 instructions for coating both High-Bind and Standard MULTI-ARRAY plate surfaces, 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 pre-coated with generic capture reagents such as avidin or streptavidin for immobilizing biotin-labeled antibodies or anti-species antibodies for the specific immobilization of polyclonal antibodies from crude sera. Detailed protocols for the use of these pre-coated plates are described in separate technical notes, available at www.meso-scale.com. Contact MSD Technical Support for additional information regarding other applications for uncoated MULTI-ARRAY plates.



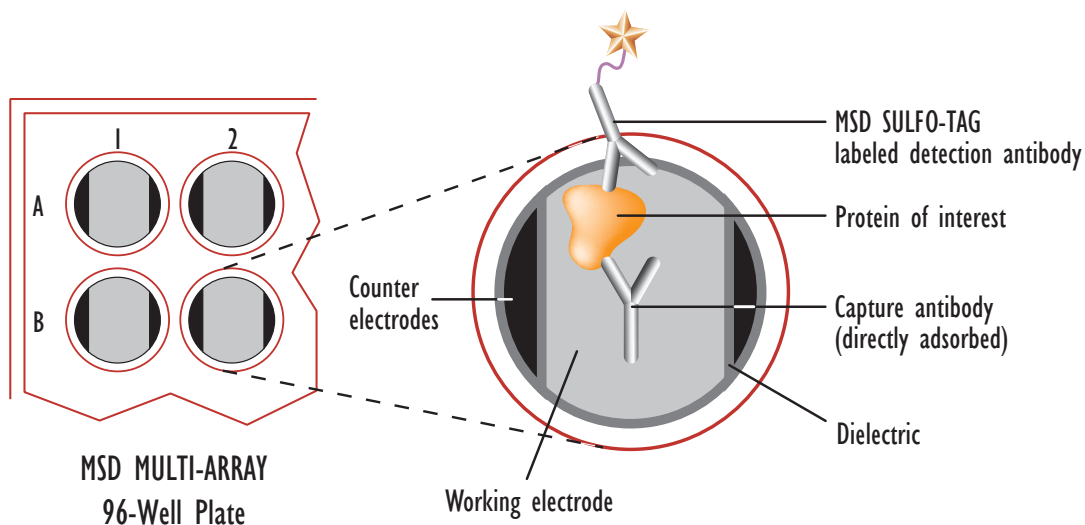
Principle of the Assay

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 MULTI-ARRAY 96-well plates supply a platform for the development of sandwich immunoassays (Figure 1). MSD provides an uncoated plate that is coated by the end user with a capture antibody against a protein of interest (target). The user then adds the sample and a solution containing a 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 antibodies immobilized on the working electrode surface; 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.



Protocol at a Glance



protocol at a glance

The following protocol describes the most conservative approach to developing highly sensitive sandwich immunoassays using 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.

Apply capture antibody in a low volume directly to electrode surface (see Sections 4 and 6 below). Allow adsorption for 1 hour at room temperature for High Bind plates, or overnight at room temperature for Standard plates (do not use a plate sealer in either case). Multiple plates can be prepared at one time and stored for future use. The detection antibody can be labeled following the instructions outlined in the MSD SULFO-TAG NHS Ester application note (see Section 4 below). One labeling preparation usually provides an adequate amount of antibody for many assays.

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

See Section 8, Detailed Protocol, for additional information.

IV Facts to Remember

facts to remember

1. Capture Antibodies

- a) For best results with direct coating, it is recommended that the capture antibody be free of carrier protein (e.g. BSA or gelatin). The presence of BSA at 5-fold molar excess (2.5-fold weight excess) compromises most assays. For antibodies with high levels of BSA, use MSD plates coated with anti species antibodies (see MULTI-ARRAY 96-Well Anti-Species Antibody Coated Plates technical note).
- b) Direct coating of the capture antibody can be performed in any buffer suitable for antibody storage, although high concentrations of glycerol (>5%) should be avoided or diluted appropriately.

2. Plates

a) Standard

- Overnight incubation for complete antibody adsorption to surface.
- Triton X-100 required in coating solution.
- IgG-saturated electrode surface is capable of binding 1 picomole of antigen from solution.

b) High Bind

- Antibody adsorption to surface complete in one hour, can be incubated overnight.
- Triton X-100 should not be used in coating solution.
- IgG-saturated electrode surface is capable of binding 5 picomoles of antigen from solution.
- More susceptible to non-specific binding; it is recommended to perform a blocking step or use optimized solutions containing blocker proteins when performing assay.

3. Detection Antibodies

- a) 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. Note that the product sheets from most vendors do not reveal if an affinity purified antibody was released from the affinity column with glycine. In these cases, there is usually sufficient residual glycine to inhibit the labeling reaction. To eliminate Tris, glycine or azide from the antibody storage solution, perform a buffer exchange using a spin column.
- b) If a purified, carrier-free antibody cannot be obtained for labeling purposes, an MSD SULFO-TAG labeled anti-species secondary antibody may be used.

V Samples

s a m p l e s

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 that offer guidance on the manipulation of different matrices are available. Please contact MSD Customer Support for more information.

VI Plate Coating Protocol

p l a t e c o a t i n g p r o t o c o l

Standard Plates

Prepare plate coating buffer:

1. Prepare a 1% Triton X-100 solution
2. Prepare plate coating buffer:
 - a) In a 15 mL tube combine:
 - 5 mL buffer of choice (e.g. PBS, TBS, HEPES)
 - 75 μ L of 1% Triton X-100 (0.015% final)

Dilute antibody to be coated:

1. Prepare 5 μ L of diluted antibody per well using plate coating buffer (with Triton).
2. In a 5 μ L volume, approximately 200 nM of antibody will saturate the surface of a Standard plate, however a titration of antibody concentrations should be explored during assay development (see Section 8).

Apply antibody to the plate:

1. Carefully apply 5 μ L of diluted antibody directly to the center of the working electrode surface being careful not to breach the dielectric.
2. The droplet should spread over time to the edge of the dielectric barrier but not cross it. (See Figure 2.)
3. Allow Standard plates to sit uncovered and undisturbed overnight.

Notes:

Triton X-100 is included in the buffer to facilitate spreading across the Standard carbon surface. It is not required when coating High Bind plates.

Coating can be performed in virtually any type of buffer appropriate for antibody storage (provided it does not contain gelatin). In some rare cases, salt can damage the antibody as the droplet dries; in these cases it may be beneficial to use buffer without added salt.

Calculation:

$$200 \text{ nM} = 200 \text{ fmol}/\mu\text{L} \times 5 \mu\text{L} = 1 \text{ pmol/well}$$

$$1 \mu\text{M} = 1 \text{ pmol}/\mu\text{L} \times 5 \mu\text{L} = 5 \text{ pmol/well}$$

It is acceptable to gently touch the electrode with the pipette tip but do not drag the tip across the surface.

To maximize sensitivity, only the working electrode surface within the MULTI-ARRAY plate should be coated. If the entire interior well surface was coated with capture species, some analyte would be captured by detection antibody on non-productive (i.e. non-light producing) areas of the well and would be lost, reducing sensitivity.

Following the overnight incubation, the plates may be used immediately, or stored at 4°C, sealed, in a dry, airtight container for up to 14 days. Note that plates manufactured by MSD have a much longer shelf life due to a specialized stabilization and packaging process.

Notes:

While the droplet will dry on the Standard carbon surface in 1-2 hours, adsorption requires an overnight incubation.

High Bind Plates

Prepare plate coating buffer:

1. To 15 mL tube add:
 - a) 5 mL buffer of choice (e.g. PBS, TBS, HEPES)

Dilute antibody to be coated:

1. Prepare 5 μL of diluted antibody per well.
2. In a 5 μL volume, approximately 1 μM of antibody will saturate the surface of a High Bind plate, however a titration of antibody concentrations should be explored during assay development (see Section 8).

Apply antibody to the plate:

1. Carefully apply 5 μL of diluted antibody directly to the center of the working electrode surface being careful not to breach the dielectric.
2. The droplet should spread over time to the edge of the dielectric barrier but not cross it. (See Figure 2.)
3. Allow High Bind plates to sit uncovered and undisturbed for at least one hour or overnight.

Following the minimum one hour incubation, the plates may be used immediately, or stored at 4°C, sealed, in a dry, airtight container for up to 14 days. Note that plates manufactured by MSD have a much longer shelf life due to a specialized stabilization and packaging process.

Notes:

Triton X-100 is included in the buffer to facilitate spreading across the Standard carbon surface. It is not required when coating High Bind plates.

Coating can be performed in virtually any type of buffer appropriate for antibody storage (provided it does not contain gelatin). In some rare cases, salt can damage the antibody as the droplet dries; in these cases it may be beneficial to use buffer without added salt.

Calculation:

$$200 \text{ nM} = 200 \text{ fmol}/\mu\text{L} \times 5 \mu\text{L} = 1 \text{ pmol/well}$$

$$1 \mu\text{M} = 1 \text{ pmol}/\mu\text{L} \times 5 \mu\text{L} = 5 \text{ pmol/well}$$

It is acceptable to gently touch the electrode with the pipette tip but do not drag the tip across the surface.

To maximize sensitivity, only the working electrode surface within the MULTI-ARRAY plate should be coated. If the entire interior well surface was coated with capture species, some analyte would be captured by detection antibody on non-productive (i.e. non-light producing) areas of the well and would be lost, reducing sensitivity.

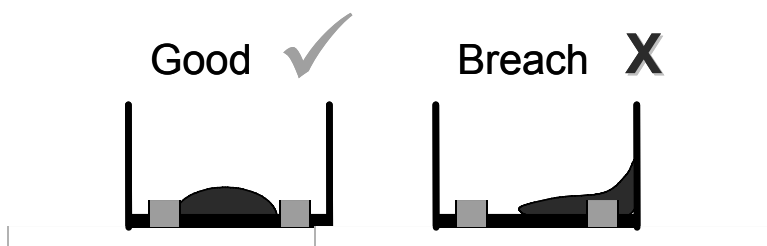


Figure 2. The droplet of diluted antibody should be confined to the working electrode surface and not breach the dielectric barrier.

VII Assay Development Experiments



assay development 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 in Sections 6 and 8, Plate Coating Protocol and Detailed Assay Protocol, respectively.

Variable	Range
Capture antibody concentration	200, 100, 50, 0 nM
Detection antibody concentration	30, 10, 3, 1 nM
Incubation format	Stepwise or simultaneous

Experimental outline:

Note: *This outline assumes that the two plates to be used in the experiment have previously been coated with capture antibody using the suggested concentrations listed above and illustrated in Figure 3 below. Please refer to Sections 6 and 8 for detailed protocol information. 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 sample and incubate.
4. Wash.
5. Add detection antibody and incubate.
6. Wash.
7. Add Read Buffer and Analyze plate.

Simultaneous Incubation format: (1 plate)

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

Addition 1: Capture Antibody (5 μ L dilute antibody solution):

	1	2	3	4	5	6	7	8	9	10	11	12
A	200 nM (30 μ g/mL)						100 nM (15 μ g/mL)					
B												
C												
D												
E	50 nM (7.5 μ g/mL)						0 nM (0 μ g/mL)					
F												
G												
H												

Addition 2: "Sample" (25 μ L in appropriate diluent):

	1	2	3	4	5	6	7	8	9	10	11	12
A	"Hi"		"Lo"		"Zero"		"Hi"		"Lo"		"Zero"	
B	or		or		or		or		or		or	
C	"Stim."		"Unstim."		"Buffer"		"Stim."		"Unstim."		"Buffer"	
D												
E												
F												
G												
H												

Addition 3: Detection Antibody (25 μ L in base buffer + 1% MSD Blocker A):

	1	2	3	4	5	6	7	8	9	10	11	12
A	30 nM (4.5 μ g/mL)											
B	10 nM (1.5 μ g/mL)											
C	3 nM (0.45 μ g/mL)											
D	1 nM (0.15 μ g/mL)											
E	30 nM (4.5 μ g/mL)											
F	10 nM (1.5 μ g/mL)											
G	3 nM (0.45 μ g/mL)											
H	1 nM (0.15 μ g/mL)											

Figure 3: Suggested plate layouts for assay development experiments.

NOTES:

VIII Detailed Assay Protocol

detailed assay protocol



Label detection antibody:

This protocol does not describe the labeling of detection antibody. This is typically performed once and sufficient 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." The base buffer should contain 0.2% Tween 20. MSD Tris Wash Buffer (1X) is an excellent choice of base buffer for phosphoprotein assays.

- 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 (e.g. Tris Wash 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 (1%) 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 (per plate):
 - 1.0 mL blocking solution
 - 2.0 mL base buffer

Note: Begin this protocol with a previously prepared plate, coated using the instructions outlined in Section 6, Plate Coating Protocol.

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 4°C and discarded after 14 days.

STEP 1

Add 150 μ L/well of blocking solution. Do not touch the pre-coated electrode surface with the pipet tip.

Incubate at room temperature for 1 hour (or at 4°C overnight for greater flexibility). 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, protease or phosphatase inhibitors may be required in the matrix or diluent.
- d) If working with purified protein, a few nanograms per well will generally provide a strong 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 three times with base buffer.

STEP 2

Dispense 25 μ L/well of diluted samples.

Incubate at room temperature, shaking, until the binding equilibrium is achieved. This usually requires 1 or more 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 5: Suggested Initial Experiment for recommended concentrations to test)

Wash plates four times with base buffer.

STEP 3

Add 25 μ L/well of detection 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 Read Buffer during this time.

Dilute Read Buffer:

- a) In a 50 mL tube combine (per plate):
 - 15 mL deionized water
 - 5 mL 4X MSD Read Buffer T, with surfactant

Wash plates three times with base buffer.

STEP 4

Carefully add 150 µL/well of diluted Read Buffer T, with surfactant, avoiding any bubbles.

Analyze with appropriate SECTOR 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.

Notes:

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

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:

IX Referenced MSD Materials



referenced MSD materials

Items	Storage
MULTI-ARRAY 96 Plates and High Bind 96 Plates	RT
MSD Read Buffer T (4X) with surfactant ¹	RT
MSD Blocker A Kit, 1L	4°C
50 g MSD Blocker A	
200 mL MSD Blocker A Buffer (5X PBS with preservatives)	
MSD SULFO-TAG NHS Ester	-20°C

Other Application Notes:

MSD SULFO-TAG NHS Ester
MSD Blocker A
MSD Read Buffers
MSD Cell Lysis Protocol

Other Technical Notes:

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

X 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.

XI Multiplex Assays

m u l t i p l e x a s s a y s



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. Antibodies must be free of carrier protein 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.

XII Support

s u p p o r t

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

Ordering Information

o r d e r i n g i n f o r m a t i o n

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