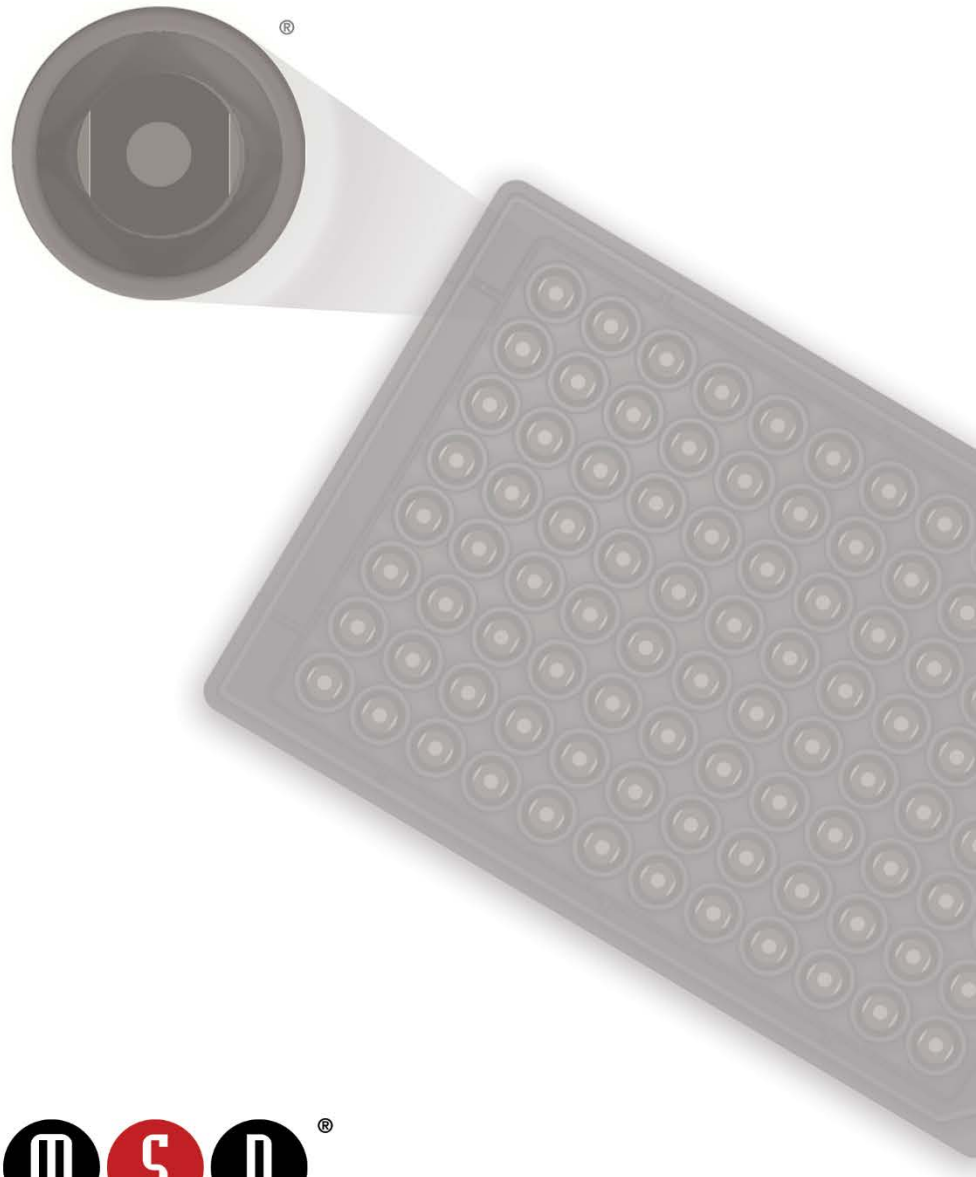
Influenza H5 Bridging Serology Assay Kit



Pack Size	Catalog No.
5-Plate Size Pack	K150AXJU

Influenza H5 Bridging Serology Kit

The Influenza H5 Bridging Serology Kit detects the presence of antibodies that bind hemagglutinin subtype 5 (H5) from the influenza A virus.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

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Contact Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 Attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

The MESO SCALE DISCOVERY® Influenza H5 Bridging Serology Assay Kit detects the presence of antibodies that bind hemagglutinin subtype 5 (H5) from the influenza A virus. This assay is intended to detect anti-H5 antibodies in the serum of multiple species, including human, rabbit, ferret, bovine, and chicken serum.

Principle of the Assay

The Influenza H5 Bridging Serology Assay Kit detects the presence of antibodies that bind to H5. The assay works by combining sample with H5 antigens that are conjugated with biotin and SULFO-TAG™ labels and provided with the kit. Anti-H5 antibodies in the sample bind to the conjugated H5 antigens to form H5 antigen-antibody complexes (see Fig. 1). This mixture is then added to the wells of a streptavidin coated plate, where the complexes are captured by the streptavidin on the plate. The plate is then read on an MSD instrument, which measures the light emitted from the MSD SULFO-TAG label.

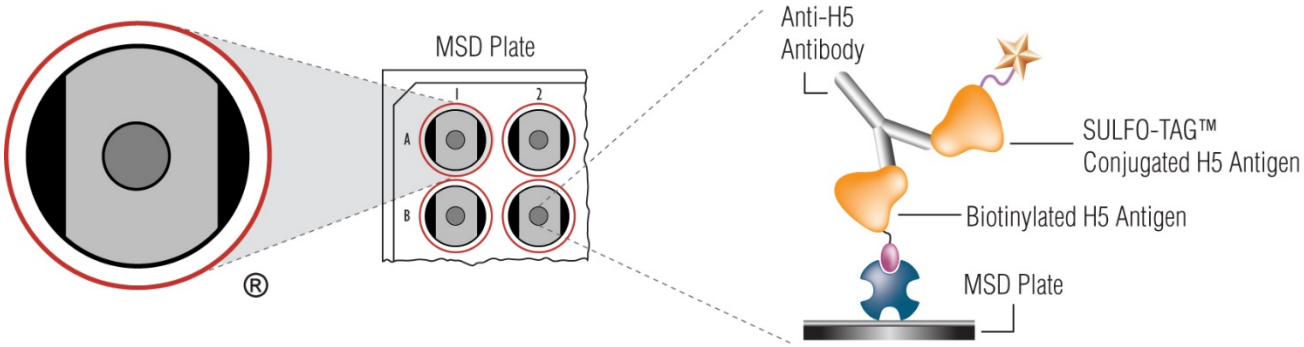

Figure 1. Small Spot plate diagram showing placement of streptavidin and the format of the Influenza H5 Bridging Serology Assay.

Materials and Equipment

Kit Components

The Influenza H5 Bridging Serology Assay Kit is available in a 5-plate size.

Table 1. Reagents that are supplied with the Influenza H5 Bridging Serology Assay Kit

Reagent	Storage	Catalog No.	Size	Quantity Supplied	Description
Biotin H5 Conjugated Protein	≤ -70C	C20AXJ-3	300 µL	1	Biotin conjugated protein
SULFO-TAG H5 Conjugated Protein	≤ -70C	D20AXJ-3	300 µL	1	SULFO-TAG conjugated protein
H5 Control 1.1	≤ -70C	C50AXJ-1	1.75 mL	1	High positive control
H5 Control 1.2	≤ -70C	C50AXJ-2	1.75 mL	1	Low positive control
H5 Control 1.3	≤ -70C	C50AXJ-3	1.75 mL	1	Negative control
Diluent 100	2–8 °C	R50AA-2	200 mL	1	Diluent for the assay
U-bottom Microplate	RT	NA	NA	5	Plate for mixing controls, sample and mastermix
Blocker A	RT	R93BA-2	250 mL	1	Blocker for streptavidin plate
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1	Read buffer
Wash Buffer 20X	RT	R61AA-1	100 mL	1	Wash buffer

Note:

This kit does not come with an MSD-provided calibrator. For users wanting to apply quantitation to the assay, we recommend using H5 Control 1.1. See *Recommended Protocol* for instructions.

Plate Compatibility

Table 2. Plates that are supplied with the Influenza H5 Bridging Serology Assay Kit

Reagent	Storage	Catalog No.	Quantity Supplied	Instrument Compatibility	Description
MSD GOLD 96-well Small Spot Streptavidin SECTOR™ Plate	2–8 °C	L4BSA-1	5	MESO SECTOR® S 600 MESO SECTOR S 600MM MESO QuickPlex SQ 120 MESO QuickPlex SQ 120MM	96-well plate, foil sealed, with desiccant
96-well Small Spot Streptavidin QuickPlex Ultra™ Plate	2–8 °C	L4BLA-1	5	MESO QuickPlex™ Q 60MM	

Optional Reagents

The Influenza H1 Cross-Reactivity Blocker can be used to reduce false positive H5 signals caused by cross-reaction of anti-H1 antibodies in human samples with the H5 protein in the kit.

Table 3. Optional reagents that can be purchased with the H5 Bridging Serology Assay Kit

Reagent	Storage	Catalog No.	Size	Number of Vials	Description
Influenza H1 Cross-Reactivity Blocker	≤ -70C	R93BL-1	220 µL	1	A reagent used in the sample preparation step to reduce false positive anti-H5 signals caused by cross-reactive anti-H1 antibodies

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Deionized water
- ❑ 0.2 µM filter needed for Blocker A preparation
- ❑ 96-well plates
- ❑ Microtiter plate shaker capable of shaking at ~700 rpm
- ❑ Microcentrifuge tubes for making serial dilutions
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput capable of accurately dispensing 50 µL and 150 µL into a 96-well microplate
- ❑ Vortex mixer

Safety

Use safe laboratory practices and wear gloves, safety glasses, and laboratory 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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com®.

Protocol

Best Practices

Read this product insert in its entirety before using this product. In addition, follow the following best practices:

Reagent Preparation

Do Not Mix Lots	Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific certificate of conformity (COC).
Aliquot Thawed Reagents	Thaw and sub-aliquot reagents supplied with this kit.
No Partial Plates	We do not recommend using a partial plate when running this panel.
Storing Thawed Reagents	Reagents received frozen should not be left at 4ºC for more than 24 hours.
Anti-H1 Antibodies	Human serum may contain anti-H1 antibodies, some of which may be able to cross-react with the conjugated H5 Protein in this kit. Customers wishing to reduce anti-H1 cross-reactivity can use the H1 Cross-Reactivity Blocker (Catalog Number: R963BL), which is available as a separate component.

Reagent Handling

Protect Reagents from Light	Avoid prolonged exposure of the MSD SULFO-TAG labelled H5 (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 During Pipetting	Avoid bubbles in wells at all pipetting steps as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection
Use Reverse Pipetting	Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
Incubation Temperature	Assay incubation steps should be performed at 20-26 ºC to maximize consistency in signals between runs.

Plate Handling

Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 500-1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
Multichannel Pipette Washing	When performing manual plate washing using a multi-channel pipette, plates should be washed using at least 150 µL of wash buffer per well. Excess residual volume after washing should be removed by gently tapping the plate on a paper towel.
Do Not Dry After Washing	Do not allow plates to dry after washing steps. Solutions associated with the next assay step should be added to the plate immediately after washing.

Plate Reading

Read Buffer at Room Temperature	Make sure that the read buffer is at room temperature when adding to the plate.
Interplate Precision	To improve interplate precision, keep time intervals consistent between adding read buffer and reading the plate.
Read Plate As Soon As Possible	Read the plate as soon as possible after adding read buffer.
Do Not Shake Plate	Do not shake the plate after adding read buffer.
Remove Plate Seal	Remove the plate seals before reading the plate.

Recommended Protocol

Bring all plates, controls and diluents to room temperature. Thaw samples on ice.

A sample plate layout is shown in Figure 3 (below).

Prepare Blocker A Solution

Follow the preparation procedure in the product insert provided with the Blocker A Kit to prepare the Blocker A solution. You may store unused Blocker A solution according to the instructions in kit components section.

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution. Dilute the stock solution before use. PBS + 0.05% Tween-20 can be used as an alternative to MSD Wash Buffer.

For one plate, combine:

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

STEP 1: Prepare Master Mix Solution

Labelled antigen is provided as a 100X stock solution. The working solution is 1X. Each plate requires 5 mL.

To prepare a 1X solution of Master Mix, combine:

- ❑ 50 µL of SULFO-TAG H5 Conjugated Protein
- ❑ 50 µL of BIOTIN H5 Conjugated Protein
- ❑ 4,900 µL of Diluent 100

Sample Preparation:

Prepare the samples by diluting with Diluent 100. The optimal dilution for samples should be determined empirically by the user. Typically, serum samples are tested at a dilution of 20-fold. Dilutions as high as 1,000-fold may be appropriate for hyperimmune samples. To allow accurate and meaningful comparison between samples, compare results obtained using the same sample dilution.

This protocol provides guidance for preparing 20-fold and 1000-fold dilutions.

1. To make a 1:20 dilution in a 2 mL tube, or 96-well plate, combine:
    - ❑ 10 µL of sample
    - ❑ 190 µL of Diluent 100
2. To make a 1:1,000 dilution in 2 mL tubes, or a 96-well plate:
    - ❑ Make a 1:50 dilution by combining 10 µL of sample with 490 µL of Diluent 100
    - ❑ Make a final, 1:20 dilution in Diluent 100 as described in Step 1

Control Preparation:

The kit includes H5 Controls, which are intended to be used undiluted. Controls are supplied without an assigned concentration.

Notes:

- This kit does not come with an MSD-provided calibrator. For users wanting to apply quantitation to the assay, we recommend using H5 Control 1.1 to generate a 7-point standard curve with 4-fold serial dilution steps using Diluent 100, along with a diluent-only blank. Users applying quantitation may assign their own concentration in arbitrary units.
- Control concentrations are lot-specific. Users applying quantitation to the assay should implement a control bridging strategy to maintain consistency between lots.

STEP 2: Combine Sample, Controls and Master Mix

- ❑ Add 40 µL/well of Master Mix solution to the U-bottom clear polypropylene plate supplied with the kit.
- ❑ Add 40 µL/well of diluted samples or controls to the same plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 1 hour.

During this time, prepare the streptavidin plate.

STEP 3: Prepare Streptavidin Plate

- ❑ Remove the plate from its packaging.
- ❑ Add 150 µL/well of Blocker A solution to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for at least 30 minutes.

STEP 4: Transfer Solution to Streptavidin Plate

After the Blocker A incubation step, wash the streptavidin plate 3 times with at least 150 µL/well of 1X MSD Wash buffer.

- ❑ Transfer 50 µL/well of the blend in the U-bottom plate to the washed streptavidin plate.
- ❑ Seal the streptavidin plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 1 hour.

STEP 5: Read Buffer Addition

After the final incubation step, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash buffer.

MSD provides MSD GOLD™ Read Buffer B ready for use. Do not dilute.

- ❑ Add 150 µL/well of the MSD GOLD Read Buffer B. **Do not shake** the plate after adding read buffer.
- ❑ Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate. Read the plate immediately after adding read buffer.

STEP 5: Analysis of Results

Users may wish to determine whether samples tested in this kit show evidence of H5 seroconversion. In such cases, users should empirically determine appropriate cut-offs to separate negative and positive results.

Users who choose to prepare a standard curve from the high control to calculate antibody concentration in samples can back-fit the measured signals for samples to the standard curve. Correcting for dilution provides the final antibody concentrations in undiluted samples. For example, if 100-fold diluted samples are tested, multiply the back-fitted concentrations by 100.

Notes:

- Standard curves used to calculate antibody concentrations can be established by fitting the signals from the diluted high control to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The best quantification of unknown samples is achieved by generating a standard curve for each plate using a minimum of two replicates at each calibrator level.
- To allow accurate and meaningful comparison between samples, compare results obtained using the same sample dilution.
- To analyze plate data generated with this kit with Methodical Mind Enterprise™ software, set up a custom Assay Method. See the *Custom Assay Methods Quick Guide* for instructions.

Additional Information

Appendix A: Signal Range of Controls

The signal range for each control is listed below.

Table 4. Signal range for each control

Reagent	Catalog No.	Signal range (Low-High)
H5 Control 1.1	C50AXJ-1	490,000-920,000
H5 Control 1.2	C50AXJ-2	47,000-85,000
H5 Control 1.3	C50AXJ-3	≤ 180

Protocol at a Glance

Note: Bring all plates, frozen reagents and Diluent 100 to room temperature. Thaw samples on ice.

- ❑ Incubate sample and Master Mix in the U-bottom polypropylene plate for 1 hr.
- ❑ Add Blocker A solution to streptavidin plate; incubate for at least 30 minutes, wash.
- ❑ Transfer 50 µL of solution from Master Mix plate to streptavidin plate; incubate for 1 hour, wash.
- ❑ Add Read Buffer and analyze plate.

Catalog Numbers

Table 5. Catalog Number for the Influenza H5 Serology Bridging Assay

Kit Name	Catalogue Number SECTOR Plate	Catalogue Number QuickPlex Ultra Plate
Influenza H5 Serology Bridging Assay	K50AXJU-2	K50AXJU-22

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Control 1.1		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
B	Control 1.2		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
C	Control 1.3		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	
D	Sample-01		Sample-09		Sample-17		Sample-25		Sample-33		Sample-41	
E	Sample-02		Sample-10		Sample-18		Sample-26		Sample-34		Sample-42	
F	Sample-03		Sample-11		Sample-19		Sample-27		Sample-35		Sample-43	
G	Sample-04		Sample-12		Sample-20		Sample-28		Sample-36		Sample-44	
H	Sample-05		Sample-13		Sample-21		Sample-29		Sample-37		Sample-45	

Figure 2. Sample plate layout that can be used for the assay.

Plate Diagrams

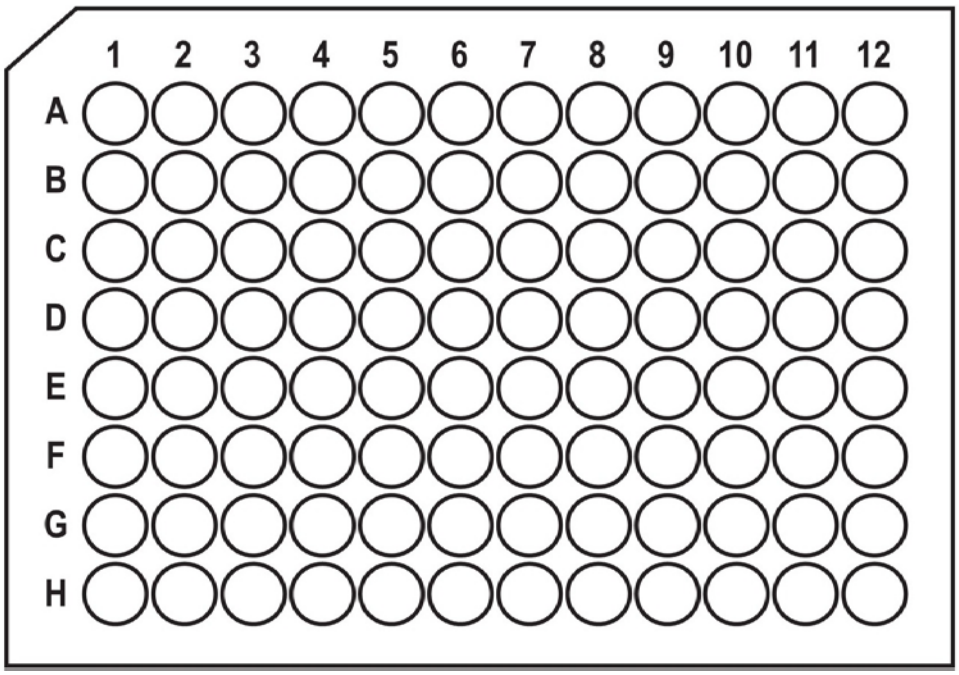

Figure 3. Plate diagram.