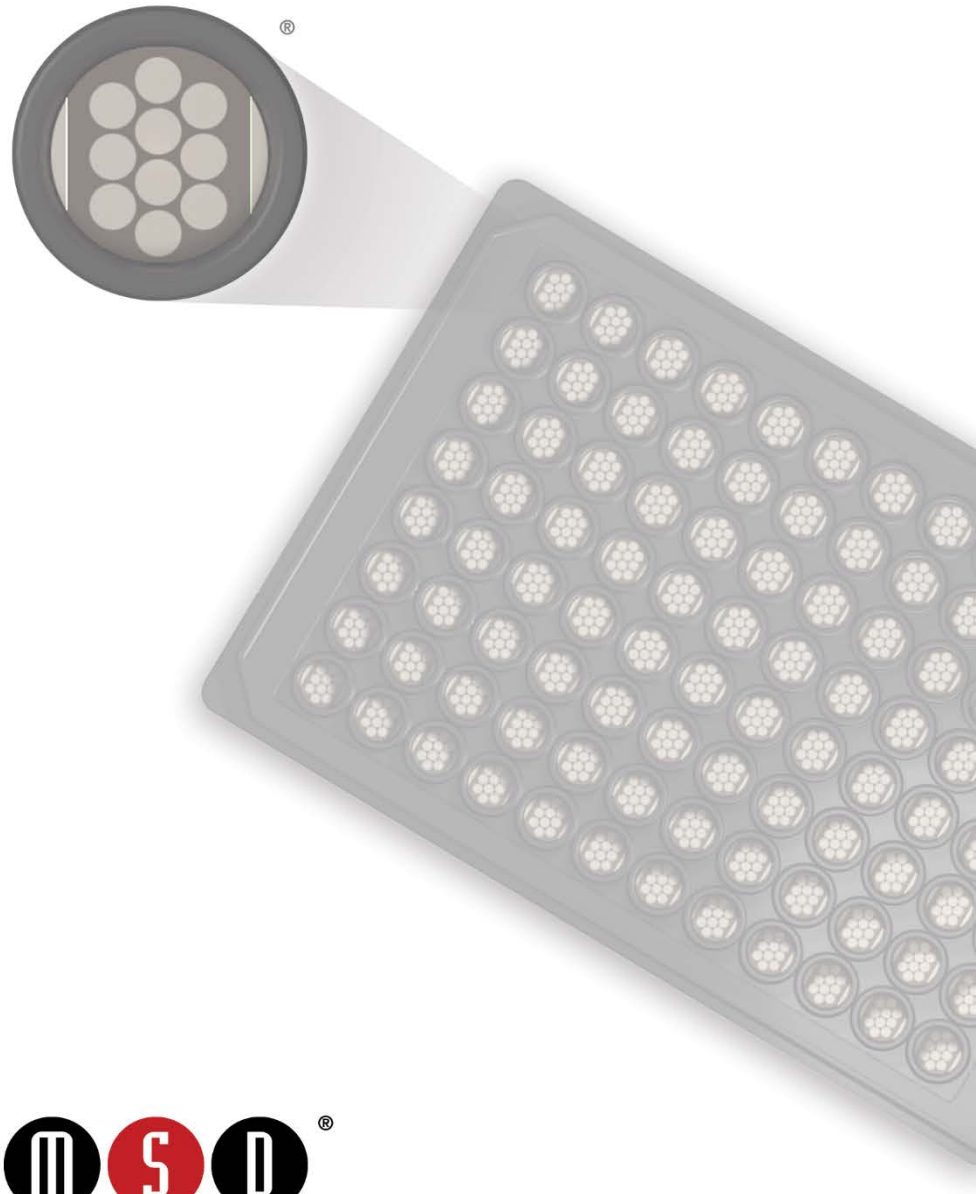
MSD® MULTI-SPOT Assay System

Orthopoxvirus Serology Kit

V-PLEX®



MSD®

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V-PLEX® Orthopoxvirus Serology Kit

The V-PLEX Orthopoxvirus Serology Kit includes a multiplex panel to detect antibodies to antigens from mpox and vaccinia viruses.

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

The MESO SCALE DISCOVERY® V-PLEX Orthopoxvirus Serology Kit measures the presence and amount of antibodies to mpox and vaccinia viral proteins. The kit is available as a panel and detects the IgG isotype of antigen-specific antibodies.

Principle of the Assay

The V-PLEX Orthopoxvirus Serology Kit quantitatively measures antibodies to mpox and vaccinia viral proteins (Tables 1 and 2). Plates are provided with 10 viral antigens (5 mpox proteins and their 5 orthologous vaccinia proteins) on spots in the wells of a 96-well plate (Figure 1). Antibodies in the sample bind to the antigens on the spots, and anti-human IgG antibodies conjugated with MSD SULFO-TAG™ are used for detection. The plate is read on an MSD instrument, which measures the light emitted from the MSD SULFO-TAG.

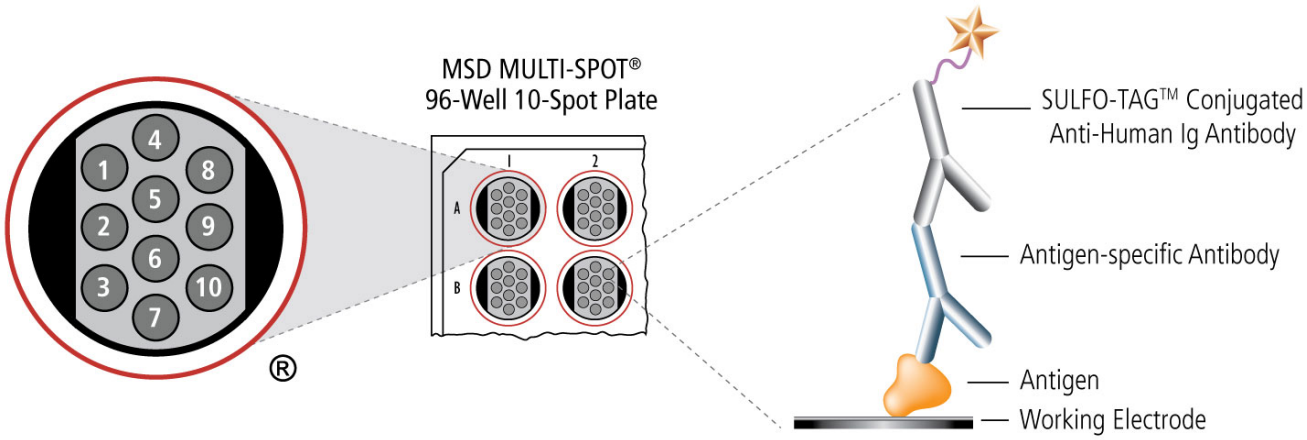

Figure 1. Schematic for the V-PLEX Orthopoxvirus Serology Kit.

Panel Components

The V-PLEX Orthopoxvirus Serology Kit is defined by a set of antigens coated on a 10-spot MULTI-SPOT® 96-well plate. A panel includes a calibrator for quantitation, controls, plate(s), detection antibodies (anti-human IgG), and all other reagents necessary to conduct the assay.

Table 1 describes the provided plates and the location of antigens on each plate. Table 2 shows the plate included in the kit. Table 3 provides a list of components included in the panel. Table 4 provides additional information about the antigens coated on the plate. The table is organized to identify the pairs of orthologous mpox and vaccinia proteins.

Table 1. List of antigens and their spot assignments on the MULTI-SPOT 96-Well, 10-Spot plates

Plate Description	Orthopoxvirus Plate 1
Spot 1	VACV A27L
Spot 2	VACV A33R
Spot 3	VACV B5R
Spot 4	VACV D8L
Spot 5	VACV L1R
Spot 6	MPXV M1R
Spot 7	MPXV E8L
Spot 8	MPXV B6R
Spot 9	MPXV A35R
Spot 10	MPXV A29L

Table 2. Antigen plates included in the V-PLEX Orthopoxvirus Serology Kit

Panel	Plate Included
Orthopoxvirus Panel 1	Orthopoxvirus Plate 1

Table 3. Reagents and Components

Reagent	Storage	Catalog Number	Size	Quantity Supplied	
				5-Plate Kit	25-Plate Kit
Orthopoxvirus Plate 1, 96-Well 10-Spot SECTOR™ Plate	2–8 °C	—	10-Spot	5 plates	25 plates
Orthopoxvirus Plate 1, 96-Well 10-Spot QuickPlex Ultra® Plate	2–8 °C	—	10-Spot	5 plates	25 plates
SULFO-TAG Anti-Human IgG Antibody (200X)♦	2–8 °C	D21ADF-3	200 µL	1 vial	5 vials
Diluent 100	2–8 °C	R50AA-2	200 mL	1 bottle	5 bottles
MSD Wash Buffer (20X)	RT	R61AA-1	100 mL	1 bottle	5 bottles
Blocker A	RT	R93BA-2	250 mL	1 bottle	5 bottles
MSD Phosphate Buffer (5X)	RT	R93SA-2	50 mL	1 bottle	5 bottles
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottles
Microplate Adhesive Film	RT	—	—	15 sheets	75 sheets
Orthopoxvirus Serology Calibrator 1	≤–70 °C	C0686-2	150 µL	1 vial	5 vials
Orthopoxvirus Serology Control Pack*		C4686-1			
Orthopoxvirus Serology Control 1	≤–70 °C		1 mL	1 vial	5 vials
Orthopoxvirus Serology Control 2	≤–70 °C		1 mL	1 vial	5 vials
Orthopoxvirus Serology Control 3	≤–70 °C		1 mL	1 vial	5 vials

RT = room temperature

* Orthopoxvirus Serology Controls 1 and 2 are positive controls, and Control 3 is a negative control

♦ IgG detection antibody used in the kit is a mouse monoclonal antibody

Dash (—) = not applicable

Table 4. Additional information about the antigens included in the V-PLEX Orthopoxvirus Serology Kit

Orthologous Pairs	Antigens	Antigen Descriptions	GenBank Protein	GenBank Nucleotide
VACV A27L / MPXV A29L	VACV A27L	Vaccinia virus A27L protein	ABD52635	DQ121394.1
	MPXV A29L	Mpox virus A29L protein	URK20577	ON563414.3
VACV A33R / MPXV A35R	VACV A33R	Vaccinia virus A33R protein	ABD52644	DQ121394.1
	MPXV A35R	Mpox virus A35R protein	URK20584	ON563414.3
VACV B5R / MPXV B6R	VACV B5R	Vaccinia virus B5R protein	ABD52686	DQ121394.1
	MPXV B6R	Mpox virus B6R protein	URK20605	ON563414.3
VACV D8L / MPXV E8L	VACV D8L	Vaccinia virus D8L protein	ABD52586	DQ121394.1
	MPXV E8L	Mpox virus E8L protein	URK20542	ON563414.3
VACV L1R / MPXV M1R	VACV L1R	Vaccinia virus L1R protein	ABD52554	DQ121394.1
	MPXV M1R	Mpox virus M1R protein	URK20517	ON563414.3

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

Calibrator and controls contain human serum and are biosafety level 2 (BSL-2) products, which should be considered potentially infectious. Appropriate precautions should be used when handling these materials. 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.

Best Practices

- Mixing or substituting reagents from different sources or different lots is not recommended.
- Assay incubation steps should be performed at 20–26 ºC to maximize consistency in signals between runs.
- Avoid prolonged exposure of the 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 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 and do not blow out residual liquid to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate 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.
- Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
- Avoid excessive drying of the plate during washing steps, especially if working inside a laminar flow hood or other high air-flow environment. Add solutions to the plate immediately after washing.
- Read buffer should be at room temperature (20-26 ºC) prior to adding it to the plate.
- Keep time intervals consistent between addition of read buffer and reading the plate to improve inter-plate precision. It is recommended that an MSD instrument be prepared to read a plate before adding Read Buffer. Unless otherwise directed, read the plate as soon as possible after adding read buffer.Do not shake the plate after adding read buffer.
- Ensure that the reagents for the next step are prepared before washing the plates in order to prevent the plates from drying out.
- Remove the plate seal before reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- We do not recommend attempting to use a partial plate when running this panel.

Recommended Protocol

Bring all plates and diluents to room temperature. Thaw samples, calibrator, and controls on ice. Thawed calibrator and controls should be equilibrated to room temperature before loading into the plates.

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 the Blocker A product insert available at www.mesoscale.com.

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

Assay and Antibody Diluent

Use Diluent 100 as assay and antibody diluent.

STEP 1: Prepare 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.

During this time, prepare calibrators, controls, and samples.

Calibrator Preparation

The kit includes a serum-based calibrator, Orthopoxvirus Serology Calibrator 1, which is used to establish a calibration curve in the assay. The calibration curve is used for calculating the concentration of human IgG against multiple antigens in the Orthopoxvirus Serology Kit.

We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw Orthopoxvirus Serology Calibrator 1 on ice, equilibrate to room temperature, and then add to Diluent 100 to make the calibrator curve solutions.

CAL-01 Preparation: Reference Standard 1, 10-fold dilution:

Prepare the highest calibrator solution (CAL-01) by diluting Orthopoxvirus Serology Calibrator 1 **10-fold**, as shown below (Figure 2):

- ❑ Add 20 µL of the Orthopoxvirus Serology Calibrator 1 to 180 µL of Diluent 100. Vortex briefly to mix. Label the vial as CAL-01.

CAL-02 to CAL-08 Preparation:

To prepare 7 calibrator solutions for the assay, plus a zero calibrator for up to 2 replicates, perform the following:

- ❑ Prepare the next calibrator (CAL-02) by adding 50 µL of CAL-01 to 150 µL of Diluent 100. Vortex briefly to mix.
- ❑ Repeat 4-fold serial dilutions (50 µL previous calibrator into 150 µL Diluent 100) to generate CAL-03 through CAL-07.
- ❑ Use Diluent 100 as the blank (CAL-08).

Note: Stock calibrator is expected to be stable for 10 years from the date of manufacture when stored at ≤–70 °C. Excess diluted calibrator should be discarded after use. For the lot-specific concentration of IgG antibody against each antigen, refer to the calibrator COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.

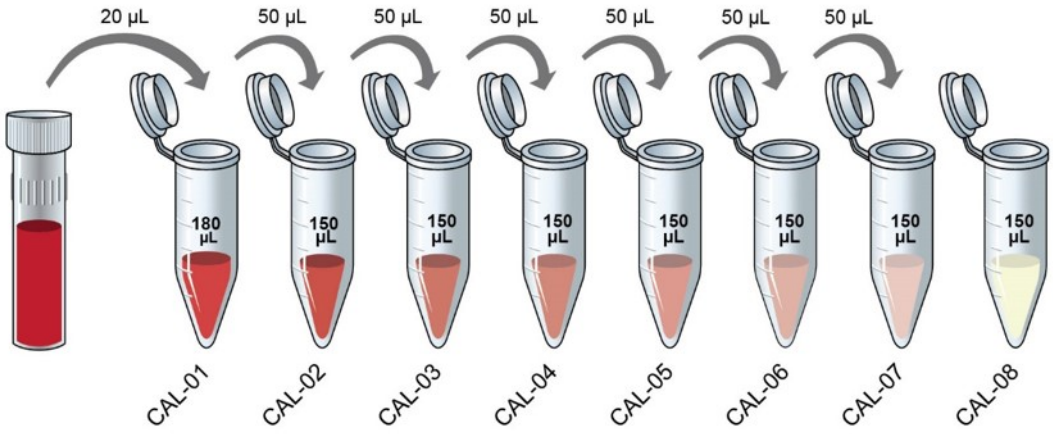

Figure 2. *Dilution schema for the preparation of calibrator solutions using a 10-fold dilution of the Orthopoxvirus Serology Calibrator 1 to generate CAL-01.*

Control Preparation

The Orthopoxvirus Serology Controls consist of three levels of controls—two positive and one negative. Positive controls have assigned concentrations of human IgG against antigens in the Orthopoxvirus Serology Kit. Controls 1 and 2 are positive controls, whereas Control 3 is a negative control. Refer to the lot-specific COA supplied with the kit for the assigned values in MSD arbitrary units (AU/mL). You can also find a copy of the COA at www.mesoscale.com.

Note: Each control is supplied at the working concentration. Do not dilute prior to use.

Thaw the control on ice and equilibrate to room temperature. Vortex briefly and spin down before loading controls into the plate.

Note: Stock control is expected to be stable for 5 years from the date of manufacture when stored at ≤–70 °C.

Sample Preparation

Prepare the samples by diluting with Diluent 100. The optimal dilution for serum and plasma samples should be determined empirically by the user. Typically, samples are measured at a dilution between 100-fold and 500-fold. Lower dilutions keep negative or low samples in the measurable range; higher dilutions prevent saturation of signal with strongly positive samples. This protocol provides guidance for preparing 100-fold and 500-fold dilutions, common choices in epidemiological studies.

This protocol provides guidance for preparing both a 100-fold and 500-fold diluted sample.

- ❑ To make an intermediate 1:10 dilution in a 2 mL tube or 96-well plate, combine:
  - o 10 µL of sample
  - o 90 µL of Diluent 100
- ❑ To make a 1:100 dilution in a 2 mL tube or 96-well deep well plate, combine:
  - o 15 µL of the 1:10 dilution from Step 1.
  - o 135 µL of Diluent 100
- ❑ To make a 1:500 dilution in a 2 mL tube or 96-well deep well plate, combine:
  - o 30 µL of the 1:100 dilution from Step 2.
  - o 120 µL of Diluent 100

STEP 2: Calibrators, Controls, and Sample Addition

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

- ❑ Add 50 µL/well of diluted samples, calibrators, and controls to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 2 hours.

During this time, prepare the detection antibody solution.

Detection Antibody Solution Preparation

Detection antibody is provided as a 200X stock solution. The working solution is 1X. You will need 6 mL per plate.

To prepare a 1X solution of detection antibody, combine:

- ❑ 5,970 µL of Diluent 100
- ❑ 30 µL of 200X SULFO-TAG anti-human IgG Antibody

STEP 3: Detection Antibody Addition

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

- ❑ Add 50 µL/well of 1X detection antibody solution to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 1 hour.

STEP 4: Read Buffer Addition

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

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

- ❑ Add 150 µL/well of MSD GOLD Read Buffer B to the plate.
- ❑ 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. Do not shake the plate after adding read buffer.

STEP 5: Analysis of Results

Calibration curves used to calculate antibody concentrations are established by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The best measurement of unknown samples is achieved by generating a calibration curve for each plate using a minimum of two replicates at each calibrator level.

Antibody unit concentrations in controls and diluted samples are determined from their electrochemiluminescent signals by backfitting to the calibration curve.

For samples, correcting for dilution provides the final antibody concentrations in undiluted samples (in AU/mL). For example, if 500-fold diluted samples are tested, multiply the backfitted concentrations by 500.

Positive controls 1 and 2 are provided pre-diluted for ease of use. Their assigned concentrations reflect the antibody concentrations in the as-provided material. Multiplying the backfitted concentrations of the positive controls by 500 will provide dilution-adjusted concentrations (in AU/mL) that are comparable to concentrations of antibodies in undiluted serum and plasma samples.

Refer to the lot-specific COA supplied with the kit for the assigned values in MSD arbitrary units (AU/mL). You can also find a copy of the COA at www.mesoscale.com.

Protocol at a Glance

Note: Bring all plates and diluents to room temperature. Thaw samples, calibrator, and controls on ice. Thawed calibrator and controls should be equilibrated to room temperature before loading into the plates.

- ❑ Add Blocker A solution; incubate with shaking for at least 30 minutes, wash.
- ❑ Add samples, calibrators, and controls. Incubate with shaking for 2 hours and wash.
- ❑ Add Detection Antibody solution. Incubate with shaking for 1 hour and wash.
- ❑ Add Read Buffer and analyze plate.

Plate Layout

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

Figure 3. Sample plate layout that can be used for the assay. Each sample, control, and calibrator is measured in duplicate in side-by-side wells.

Appendix A: Analytical Sensitivity

Limits of quantification (LOQs) were estimated based on the Orthopoxvirus Serology Calibrator 1 performance over multiple runs and multiple lots. The table below shows the estimated in-well quantitative range for each assay.

Multiplying the LLOQ and ULOQ values in the table by the sample dilution factor will provide dilution-adjusted limits of quantification.

Table 5. LLOQ and ULOQ Concentrations in MSD (AU/mL)

Antigens	IgG	
	LLOQ and ULOQ concentration in MSD arbitrary units (AU/mL)	
	LLOQ	ULOQ
VACV A27L	0.028	25.8
VACV A33R	0.026	89.8
VACV B5R	0.025	18.9
VACV D8L	0.028	17.6
VACV L1R	0.039	9.83
MPXV M1R	0.022	8.56
MPXV E8L	0.025	11.8
MPXV B6R	0.058	14.2
MPXV A35R	0.021	60.1
MPXV A29L	0.033	20.4

Appendix B: Analytical Specificity

To assess specificity, the mpox and vaccinia viral antigens coated on the plate were tested for nonspecific binding using commercially available monoclonal and polyclonal antibodies against mpox antigens. As expected, ortholog antigens between mpox and vaccinia virus showed varied cross-reactivity. No other cross-reactivity was observed within the mpox and vaccinia virus antigens.

Observed cross-reactivity: 99.2% between MPXV M1R and VACV L1R; 43.4% between MPXV E8L and VACV D8L; 84.2% between MPXV B6R and VACV V5R; 198% between MPXV A35R and VACV A33R; and 73.2% between MPXV A29L and VACV A27L.

Note: The reactivity towards the ortholog antigens and cross-reactivity between orthologs is sample dependent.

Catalog Numbers

Table 6. Catalog number for the V-PLEX Orthopoxvirus Serology Kit

Kit Name	IgG	
	5-Plate Kit	25-Plate Kit
V-PLEX Orthopoxvirus Panel 1 Kit	K15688U-2	K15688U-4
V-PLEX Orthopoxvirus Panel 1 Kit, QuickPlex®	K15688U-22	K15688U-24

Plate Diagram

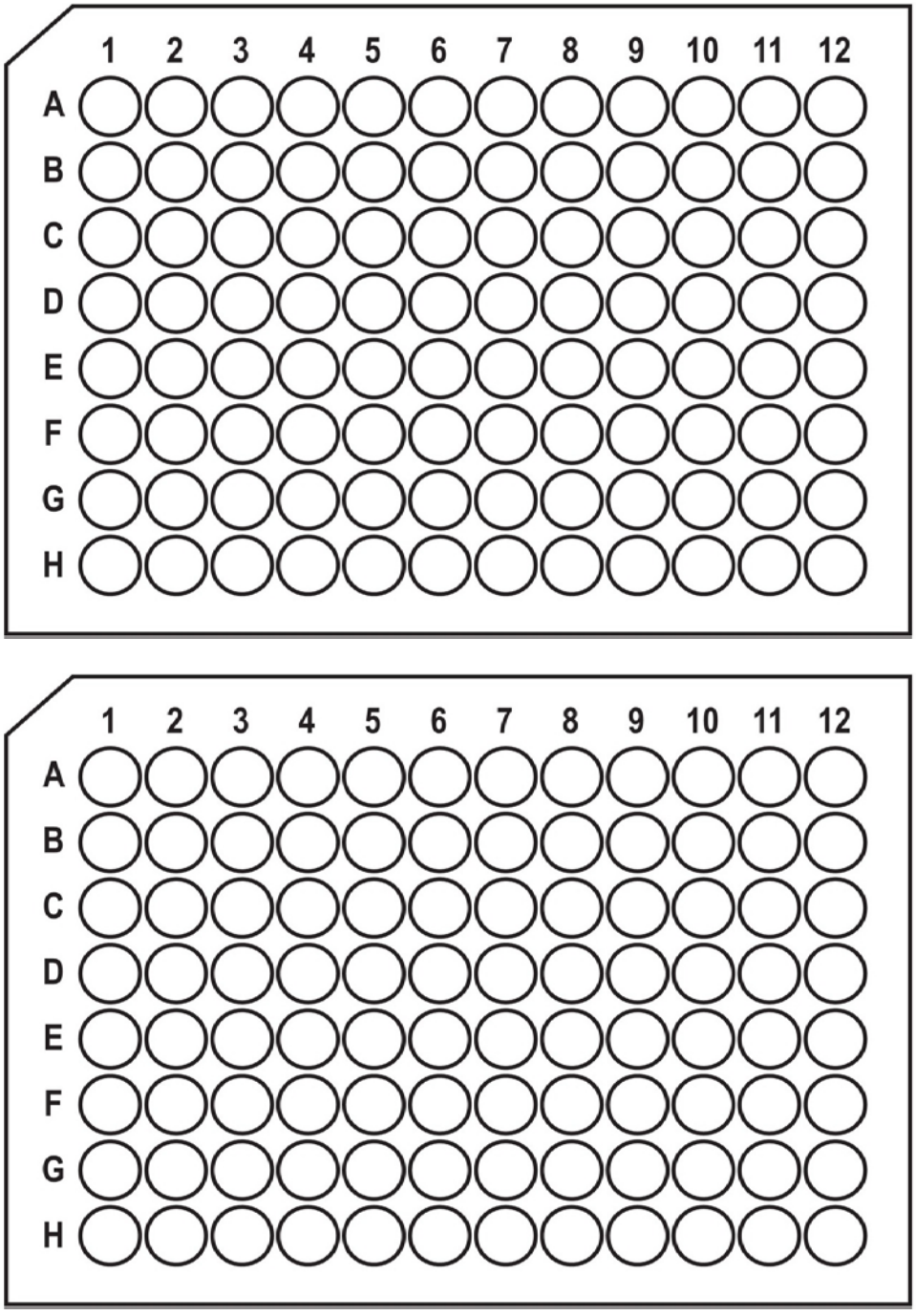

Figure 4. Plate diagram.