



**MOCKV
SOLUTIONS**

MVM-MVP Kit

Product Guide

Cat. No.: C1219

Version G March 2020

PRECAUTIONS

**FOR RESEARCH USE ONLY
NOT FOR IN VITRO DIAGNOSTIC USE**

Prior to using Kit, carefully read all instructions.

Use universal precautions when handling kit components and test specimens.*

To avoid cross-contamination, use separate pipet tips for each specimen.

When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.

Wear protective clothing and eyewear.

*from MMWR, June 24, 1988, Vol. 37, No. 24, pp. 377-382, 387-388.

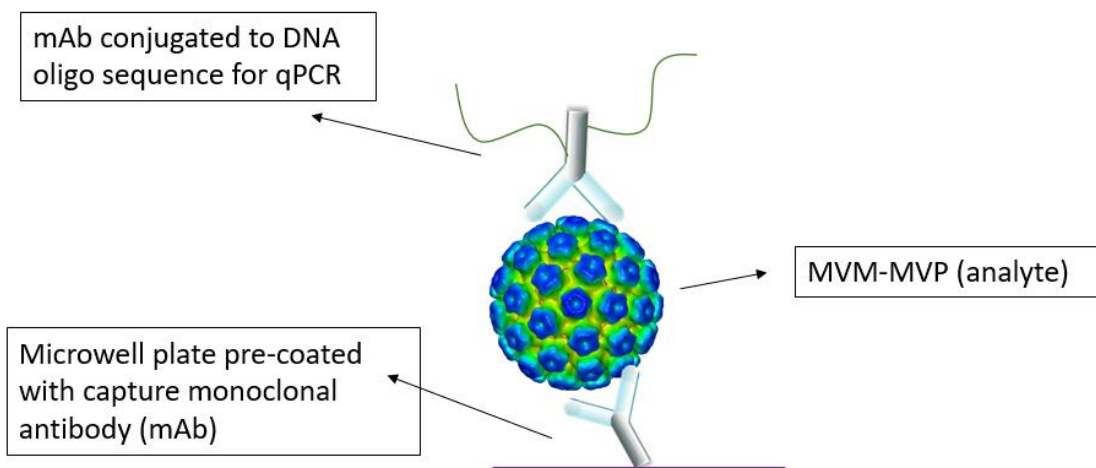
PRINCIPLE OF THE KIT

This assay is an “Immuno-qPCR” assay. It is a two-part assay which consists of an Immunoassay step and a qPCR step.

Immunoassay Step - Samples or standards are added to the wells of the microwell plate where MVP, if present, binds to the immobilized anti-MVP antibody. After washing, a DNA conjugated anti-MVP antibody is then added which will bind to the MVP (Figure 1). Following another wash sequence, a “dissociation” buffer is added to each well and after a brief incubation, samples are micropipetted into a qPCR plate for the qPCR portion of the assay.

qPCR Step – Taqman qPCR utilizing primers and probe specific to the conjugated DNA is conducted.

Figure 1: Immunoassay Step – MVP Complex



REAGENTS

Materials Supplied:

Immuno-Assay Reagents (Store at 4°C)

- C1201: mAb Coated Microplates (3 plates)
- C1202: Spiking MVP (1E12 MVP/mL), 1 x 1.5 mL's
- C1218: anti-MVP mAb conjugate (Lyophilized): 6 glass vials
- C1220: Assay Diluent (5X): 1 x 30 mL's
- C1206: Plate Wash Buffer 1 (20X), 3 x 50 mL's:
- C1217: Plate Wash Buffer 2 (20X): 1 x 20 mL's
- C1208: Sample Recovery Buffer (1X): 1 x 30 mL's

qPCR Reagents (Store at -20°C)

- C1209: 2X Master Mix (2X), clear-cap tube: 3 x 1.5 mL's
- C1210: Forward Primer (1X), green-cap tube: 450 μ L's
- C1211: Reverse Primer (1X), orange-cap tube: 450 μ L's
- C1212: 6-FAMTM Probe (1X), purple-cap tube: 300 μ L's
- C1213: Nuclease-Free Water, clear-cap tube: 1 x 2.0 mL

Materials Required but Not Supplied:

- Test tubes and rack for preparing specimen and control dilutions
- Bottles for preparation of 1X reagents
- Distilled or deionized water
- qPCR Plates and qPCR plate sealers

Equipment Required but Not Supplied:

- Adjustable micropipettes (single and multichannel)
- Water bath
- Scale
- Thermocycler with qPCR capabilities
- Centrifuge with adaptor to spin microwell plates
- Timer

RECOMMENDATIONS

Storage:

- Store Spiking MVP at 2°- 8°C
- Store all Immunoassay kit reagents at 2°- 8°C.
- Store all qPCR kit reagents at -20°C.

Immuno-qPCR:

It is recommended that Spike/Recovery and Hold Time studies be performed in representative in-process material prior to MVP spiking studies.

Spike/Recovery

High concentrations of therapeutic product, impurities or buffer components present in samples may inhibit the binding of MVP to anti-MVP antibodies during Immuno-qPCR. When such product or sample matrix interference can be demonstrated, it may be necessary to dilute samples to a Minimal Required Dilution (MRD) to achieve an acceptable recovery. Spike/Recovery experiments should be performed on each unique sample matrix ahead of time and can be accomplished as exemplified below:

1. Prepare 1 mL of each sample matrix “neat”, 1:2 and 1:10 in PBS.
2. Prepare 1 mL of PBS
3. Spike MVP into each preparation from 1 and 2 (Target: 5.0×10^7 MVP's/mL)
4. Prepare Controls
 - a. 1 mL of unspiked, undiluted matrix
 - b. 1 mL of unspiked PBS

- Run all samples from above along with a MVP dilution series according to Instructions provided in the Immuno-qPCR User Manual.
- Determine % recovery relative to spiked PBS result. If necessary, determine MRD. *Acceptable recovery is defined as between 50-150%.*

Hold Studies

Certain impurities (ex. proteases) or strongly acidic/basic buffer conditions may affect the long-term stability of MVP's in samples collected during experimentation from the time they are collected to the time they are analyzed via Immuno-qPCR. If a long duration of time between collection and analysis is anticipated, freezing samples at -80°C is recommended. As an added precaution, Hold Time experiments could be performed on each unique sample matrix ahead of time and can be accomplished as exemplified below:

- Spike each matrix with MVP and store according to Table 1 below.

Matrix	Target Spike Concentration	Storage	Final Amount
To each experimental Matrix	Low – 5.0×10^6 MVP/mL	-80°C	1 mL
		4°C	1 mL
	High - 5.0×10^8 MVP/mL	-80°C	1 mL
		4°C	1 mL

Table 1. Hold Study

- After 1 week, remove 1 mL -80°C and one 1 mL 4°C aliquots of each matrix from storage (both Low and High concentrations).
- Spike 0.5 mL of fresh matrix to 5.0×10^6 MVP/mL and 0.5 mL of fresh matrix to 5.0×10^8 MVP/mL.
- Run all samples from above along with a MVP dilution series according to Instructions provided in the Immuno-qPCR User Manual.

5. Analyze % concentration difference between freshly prepared samples and 1 week Hold samples. *Acceptable % change is defined as between 50-150%*

If needed, consult with MockV Solutions for advice on how to conduct and interpret Spike/Recovery and/or Hold Time experiments.

Read Immuno-qPCR section for complete instructions on reagent preparation and procedure before conducting either test or proceeding to conduct MVP spiking experiment.

MVP Spiking Study Procedure

Step 1: Prepare pre-spiked load material accordingly (ex. warm to room temperature, pre-filter, etc.) and determine volume.

Step 2: Determine desired MVP spike by:

- A) Targeting a total MVP particle challenge (ex. 11 log₁₀ Total MVP Particles), or;
- B) Targeting a final MVP particle concentration (ex. 1.0 x 10¹⁰ MVP Particles/mL of load).

Step 3: Determine volume of Spiking MVP to add into load. If spiking according to “A” above, use the following formula:

$$\text{Volume (mL)} = \frac{\text{Target Total log}_{10} \text{ MVP Challenge}}{1E12 \text{ MVP Particles/mL}}$$

If spiking according to “B” above, use the following formula:

$$\text{Volume (mL)} = \frac{(\text{Target Final MVP Concentration}) \times (\text{Target Final Load Volume})}{1E12 \text{ MVP Particles/mL}}$$

- Step 4:** Pipette the determined volume of Spiking MVP into load. Mix gently. Remove 1 mL of sample as load Sample.
- Step 5:** Process remaining amount of spiked load through desired separation technique. Collect fraction and pool as necessary and prepare 1 mL aliquots of each for analysis.
- Step 6:** Upon completion of separation technique, store load, fraction and pools samples at -80°C or 4°C until Immuno-qPCR analysis (based on results from Hold Study, described above).

PREPARATION OF REAGENTS

Assay Diluent:

To make MVP Dilution Series and anti-MVP mAb conjugate Working Solution below, dilute 6 mL of 5X Assay Diluent to 30 mL final volume using distilled or deionized water. Any diluted Assay Diluent remaining after completion of the assay may be stored at 4°C for up to one month.

MVP Dilution Series:

The MVP is provided at a concentration of 1.0E12 MVP/mL. Prepare a series of eight standards from the MVP Stock Solution according to Table 1 below. Change tip after each addition and resuspend fluid by pipetting up and down prior to each transfer.

Notes:

- Prepare Dilution series no more than 20 mins prior to adding to wells, as per procedure below.
- Using a dilution reservoir will aid in this operation (ex. VWR Cat# 490006-654)
- Do not decrease volumes for partial plate assays.
- Any diluted MVP remaining after the completion of the assay should be discarded appropriately; do not save diluted MVP Standard.

Table 1: MVP Dilution Scheme

Standard Number	MVP Stock Solution (µL)	Assay Diluent (µL)	Concentration of MVP (MVP/mL)
#1	10.0 µL of MVP Stock Soln.	990 µL	1.0E10
#2	100.0 µL of #1	900 µL	1.0E9
#3	100.0 µL of #2	900 µL	1.0E8
#4	100.0 µL of #3	900 µL	1.0E7

#5	100.0 μ L of #4	900 μ L	1.0E6
#6	500.0 μ L of #5	500 μ L	5.0E5
#7	200.0 μ L of #6	800 μ L	1.0E5
#8	Diluent (No VLP)	1000 μ L	0.00

anti-MVP mAb conjugate Working Solution:

For each **half** microplate, add 6 mL's of **1X** Assay Diluent to a lyophilized vial containing anti-MVP mAb conjugate. Gently dissolve contents. Avoid vigorous shaking or vortexing. Perform this operation at least 15 minutes prior to use.

SOLUTION WILL REMAIN MILKY BUT SHOULD BE CLEAR OF ANY CLUMPS

Notes:

- anti-MVP mAb conjugate is conjugated to template DNA which is detectable through qPCR. Take extreme caution while preparing and handling this reagent to avoid false positive signal generation. Spatial separation from other reagents is suggested during preparation and use.
- Any diluted anti-MVP mAb conjugate Working Solution remaining after completion of the assay should be treated with bleach (or similar DNA elimination treatment solution(s)) and discarded.
- Areas where anti-MVP mAb conjugate has been prepared or used should be cleaned thoroughly with bleach (or similar DNA elimination treatment solution(s)).
- Don new pair of gloves after handling anti-MVP mAb conjugate
- If possible, use separate micropipettor or clean micropipettor with hydrogen peroxide or equivalent after use.

qPCR Positive Control:

Dilute 5 μ L of anti-MVP mAb conjugate Working Solution, prepared above into 495 μ L of 1X Assay Diluent.

Plate Wash Buffer 1:

Check Buffer for precipitant prior to use and dissolve in 37°C bath if necessary

To prepare for a full plate (12 strips) dilute 50 mL's of 20X Plate Wash Buffer 1 to a final volume 1L with distilled or deionized water prior to use. Store 1X Plate Wash Buffer at room temperature for up to three (3) months.

Plate Wash Buffer 2:

Check Buffer for precipitant prior to use and dissolve in 37°C bath if necessary

To prepare for a full plate (12 strips) dilute 5 mL's of 20X Plate Wash Buffer 2 to a final volume 100 mL's with distilled or deionized water prior to use. Store 1X Plate Wash Buffer at room temperature for up to three (3) months.

Plate Washing Guidelines

During the course of the Immunoassay, multiple wash steps will occur to remove non-bound or non-specifically bound MVP and MVP Detector Antibody. When washing manually (multi-channel pipette or manifold), careful consideration should be taken to avoid cross-contamination of wells. Treat the MVP Detector Antibody as a contaminant and handle with extreme caution. qPCR is a very sensitive technique and small amounts of target present can lead to high background.

TEST PROCEDURE

Part I. IMMUNOASSAY

Estimated Time: 3 Hours

Prior to starting the assay, spatially plan out the standards and samples that will be added to the mAb Coated Microplate (Immunoassay) bearing in mind the later transfer to a qPCR plates (additional controls may be run on the qPCR plate). Typically, 3-4 replicates of each MVP Dilution Series Standard and 3-4 replicates of each experimental sample should be run. See Appendix 1 for an example plate layout.

Allow all reagents to reach room temperature before use.

Step 1: Open foil bag containing MVP Antibody Coated Microplate and remove the desired number of strips. Numerically mark each strip with a marker in case of detachment during assay. If the entire 96 well plate is not used, remove surplus strips from the plate frame. Place surplus strips and desiccant into the resealable foil bag, seal and store at 2-8°C.

Note: Plate must be warmed to room temperature prior to use. Seal foil bag containing unused strips.

Step 2: Wash each strip 3 times with ≥ 200 μL of 1X Plate Wash Buffer 1 and discard. Fill each strip with 400 μL of 1X Plate Wash Buffer 1 and allow wells to soak for > 10 min, then discard and thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells.

Note: After blotting dry proceed **quickly** to Step 3 to avoid drying.

Step 3: Pipet 100 μ L of samples or Dilution Series standards #1-8 (preparation instructions on page 10) into appropriate number of wells.

Step 4: Incubate in a 37°C water bath for 30 mins.

Step 5: Discard samples and wash plate 5 times with Plate Wash Buffer 1. For the first 3 washes, add ~200 μ L of 1X Plate Wash Buffer 1. Discard carefully to avoid cross-contamination. For the final 2 washes, add ~ 400 μ L of 1X Plate Wash Buffer 1. After final discard, thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells.

Note: After blotting dry proceed *quickly* to Step 6 to avoid drying.

Step 6: Pipet 100 μ L of MVP Detector Antibody Working Solution into each well (preparation instructions on page 11).

Step 7: Incubate in a 37°C water bath for 30 minutes.

Note: During this incubation, Steps 1-3 of Part II: qPCR should be performed (see below). See notes on page 11 regarding proper handling of MVP Detector Antibody.

Step 8: Aspirate each strip fully with a multichannel pipette (discarding into bleach carefully) and discharge tips into bleach. Add ~200 μ L of 1X Plate Wash Buffer 1 into each well.

Step 9: Repeat Step 8, two more times. Discard Plate Wash Buffer after final addition.

Step 10: Add ~ 400 μ L of 1X Plate Wash Buffer 1 into each well and discard. Repeat 9 more times. After final discard, blot with paper towels thoroughly on benchtop.

Step 11: Add 200 μ L of 1X Plate Wash Buffer 2 into each well. Discard and repeat 2 more times. After final discard, blot thoroughly with paper towels on benchtop.

Step 12: Pipet 50 μ L of Sample Recovery Buffer into all wells. Let sit for at least 5 minutes. Proceed to Step 4 of Part II: qPCR.

Strips can be held in Sample Recovery Buffer at 4°C for up to 24 hours.

PART II. qPCR

Estimated Total Time: 1 Hour

Step 1: Calculate number of samples to be evaluated. Sample number should be equal to the number of wells used in Part I of the assay plus any qPCR controls (ex. No Template Control, Positive Control, etc.).

Step 2: Prepare PCR Mix Solution according to Table 3:

Table 3: Preparation of PCR Mix Solution

Reagent	Volume/well	Total Volume Needed¹
2X Master Mix	12.5 μ L	
Forward Primer	0.75 μ L	
Reverse Primer	0.75 μ L	
6-FAM TM Probe	0.50 μ L	
DNase/RNase Free Water	5.5 μ L	

¹Multiply Volume/well for each reagent by number of required wells (factoring in additional wells for pipetting allowance).

Step 3: Pipet 20 µL of resulting solution into each well to be used of a qPCR plate

Step 4: Add 5 µL of samples collected during Part I into each well from Step 3. Add 5 µL of controls (ex. water for no template control, qPCR positive control, etc.)

Step 5: Seal qPCR plate with appropriate optical plate sealer (not provided) and centrifuge.

Step 6: Run qPCR assay using either of the following parameters:

Table 4A: Parameters for “Fast” Systems

Fast Thermal Cycler Profile				
Stage	Repetitions	Temperature	Time	Ramp Rate
1	1	50.0 °C	2:00	100
2	1	95.0 °C	2:00	100
3	40	95.0 °C	0:03	100
		60.0 °C	0:30	100

Table 4B: Parameters for Standard Systems

Standard Thermal Cycler Profile				
Stage	Repetitions	Temperature	Time	Ramp Rate
1	1	50.0 °C	2:00	100
2	1	95.0 °C	2:00	100
3	40	95.0 °C	0:15	100
		60.0 °C	1:00	100

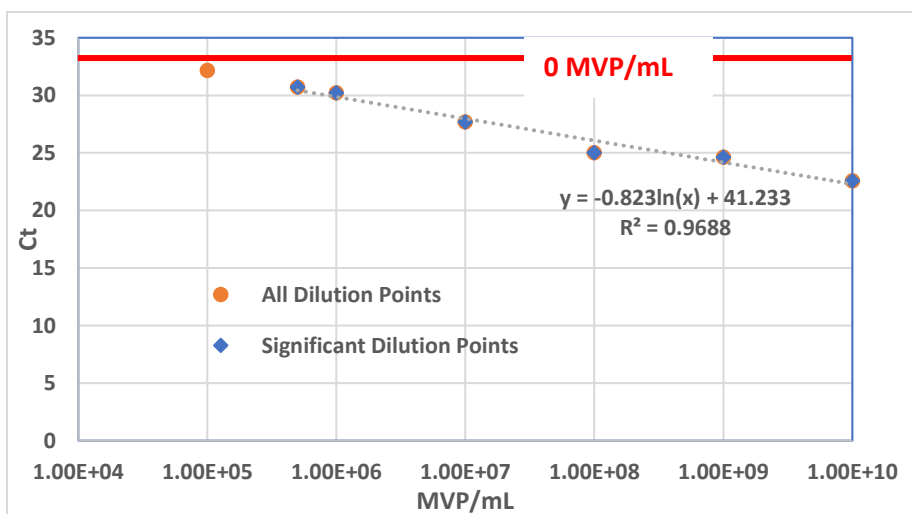
Note: Probe contains 6-FAM™ fluorescent dye (excitation 495nm, emission 520nm). Set RT-PCR system accordingly.

Calculation of Results

A dilution series of known MVP concentrations will be run alongside unknown experimental samples. From this series, a standard curve will be generated. Typically, curves are best fitted after excluding erroneous data points and eliminating low standard curve concentrations whose average Ct value(s) are not significantly (ex. > 1 standard deviation, σ) different from the 0 MVP/mL background of the assay. See Example 1 below:

Example 1: Raw data, average Ct values derived from MVP Dilution Series (Above) and Standard Curve graph with equation (below)

MVP Conc. (MVP/mL)	Ct value 1	Ct value 2	Ct value 3	Ct value 4	Avg. Ct Value	Standard Deviation (σ)	CV
1.00×10^{10}	22.65	22.93	22.64	22.09	22.58	0.35	1.55%
1.00×10^9	24.35	24.88	25.00	24.21	24.61	0.39	1.57%
1.00×10^8	25.25	25.56	25.06	24.17	25.01	0.60	2.38%
1.00×10^7	27.65	27.17	28.75	27.19	27.69	0.74	2.68%
1.00×10^6	30.27	29.56	30.98	29.98	30.20	0.59	1.97%
5.00×10^5	31.52	32.46	28.40	30.44	30.71	1.74	5.67%
1.00×10^5	31.05	33.45	31.87	32.26	32.16	1.00	3.10%
0	31.93	30.75	34.18	35.94	33.20	2.32	6.97%



Note: "Significant Dilution Points" overlay "All Dilution Points", thus only 1E5 MVP/mL point is visible.

In the example above, the 1.0×10^5 MVP/mL Dilution Series point was eliminated from the data set used to determine the standard curve. This is because the average Ct of this data point was not significantly different from the average Ct of the 0 MVP/mL data point (1.0×10^5 MVP/mL AVG + $\sigma > 0$ MVP/mL AVG).

Once a logarithmic standard curve is established, MVP concentrations of unknown samples can be back-calculated from the equation of the curve. See Example 2 below based on the standard curve equation from Example 1:

Example 2: Back-calculation MVP concentration determination of unknown samples

Sample	Ct Value	Back-Calc. Conc. (MVP/mL)
Load	22.58	6.97×10^9
Pool	32.11	6.52×10^4

Log Reduction Values (LRV) can then be calculated as shown in Equation 1 below:

$$LRV = \log_{10} \left\{ \frac{(C_l \times V_l)}{(C_p \times V_p)} \right\}$$

where C is the MVP concentration, V is the volume and l or p denote Load or Pool respectively.

Concentrations determined from pool samples may be below the assay's limit of quantification (LOQ). The assay's LOQ is the lowest significant quantitation point of the assay – a value that could be determined several ways. One way is by performing the following steps:

1. Convert Ct values from each 0 MVP/mL data point to concentration values by back calculating the Ct's against the standard curve.
2. Determine the average 0 MVP/mL concentration values from step #1 above.
3. Determine the standard deviation (σ) of concentrations determined from # 1 above and multiply this number by 3.

4. Add the average concentration value determined from step #2 to the 3σ value determined in step #3.

See Example 3 based on the data from Example 1:

	1	2	3	4
Ct Value	31.93	30.75	34.18	35.94
MVP/mL	8.06×10^4	3.37×10^5	5.24×10^3	6.16×10^2

Example 3: LOQ determination through standard deviation method

0 MVP/mL AVG	σ	3σ	AVG + 3σ
1.06×10^5	1.59×10^5	4.76×10^5	5.81×10^5

As seen, an LOQ of 5.81×10^5 MVP/mL was determined. Since this value is higher than the determined pool concentration (C_p) of Example 2, it should be substituted and used in Equation 1 to determine LRV. Assuming Load and Pool volumes of 150 mL, the LRV of Examples 1-3 would be calculated as follows:

$$LRV = \log_{10} \left\{ \frac{(6.97 \times 10^9 \times 150)}{(5.81 \times 10^5 \times 150)} \right\}$$

$$LRV = \geq 4.07$$

The \geq inequality sign signifies that since the LOQ was used to generate the result, the value reported is a minimum LRV. When the LOQ is not used in Equation 1, the value determined is absolute and does not need to be reported as an inequality.

Appendix 1:

The following is meant to serve as an example plate layout for using this Beta Kit. If space permits, it is advisable to run each standard or sample as (n=4) as opposed to (n=3).

Immuno-plate Test 1: Spike/Recovery and Hold Samples

Std 1	Std 1	Std 1	Spiked Matrix "neat"	Spiked Matrix "1:10"	Hold Sample (1 week -80c)
Std 2	Std 2	Std 2	Spiked Matrix "neat"	Spiked PBS	Hold Sample (1 week -80c)
Std 3	Std 3	Std 3	Spiked Matrix "neat"	Spiked PBS	Hold Sample (1 week 4c)
Std 4	Std 4	Std 4	Spiked Matrix "1:2"	Spiked PBS	Hold Sample (1 week 4c)
Std 5	Std 5	Std 5	Spiked Matrix "1:2"	Unspiked Matrix	Hold Sample (1 week 4c)
Std 6	Std 6	Std 6	Spiked Matrix "1:2"	Unspiked Matrix	Hold Sample control (fresh prep)
Std 7	Std 7	Std 7	Spiked Matrix "1:10"	Unspiked Matrix	Hold Sample control (fresh prep)
Std 8	Std 8	Std 8	Spiked Matrix "1:10"	Hold Sample (1 week -80c)	Hold Sample control (fresh prep)

Note: Choose a "worst case" matrix for the Spike/Recovery and Hold Sample analysis. For Hold Sample, consider spiking to 5.0E7 MVP/mL (center-point). If center-point is chosen, Hold Sample control may not be necessary (if Spiked Matrix "neat" is the same concentration)

PCR Plate Test 1: Samples from Immuno-plate + PCR Controls

Std 1	Std 1	Std 1	Spiked Matrix "neat"	Spiked Matrix "1:10"	Hold Sample (1 week -80c)	PCR Control (+)
Std 2	Std 2	Std 2	Spiked Matrix "neat"	Spiked PBS	Hold Sample (1 week -80c)	PCR Control (+)
Std 3	Std 3	Std 3	Spiked Matrix "neat"	Spiked PBS	Hold Sample (1 week 4c)	PCR Control (+)
Std 4	Std 4	Std 4	Spiked Matrix "1:2"	Spiked PBS	Hold Sample (1 week 4c)	
Std 5	Std 5	Std 5	Spiked Matrix "1:2"	Unspiked Matrix	Hold Sample (1 week 4c)	
Std 6	Std 6	Std 6	Spiked Matrix "1:2"	Unspiked Matrix	Hold Sample control (fresh prep)	NTC
Std 7	Std 7	Std 7	Spiked Matrix "1:10"	Unspiked Matrix	Hold Sample control (fresh prep)	NTC
Std 8	Std 8	Std 8	Spiked Matrix "1:10"	Hold Sample (1 week -80c)	Hold Sample control (fresh prep)	NTC

Immuno-plate Test 2: Samples

Std 1	Std 1	Std 1	Load 1	Load 2	Pool 3
Std 2	Std 2	Std 2	Load 1	Pool 2	Pool 3
Std 3	Std 3	Std 3	Load 1	Pool 2	Load 4
Std 4	Std 4	Std 4	Pool 1	Pool 2	Load 4
Std 5	Std 5	Std 5	Pool 1	Load 3	Load 4
Std 6	Std 6	Std 6	Pool 1	Load 3	Pool 4
Std 7	Std 7	Std 7	Load 2	Load 3	Pool 4
Std 8	Std 8	Std 8	Load 2	Pool 3	Pool 4

PCR Plate Test 2: Samples from Immuno-plate + PCR Controls

Std 1	Std 1	Std 1	Load 1	Load 2	Pool 3	PCR Control (+)
Std 2	Std 2	Std 2	Load 1	Pool 2	Pool 3	PCR Control (+)
Std 3	Std 3	Std 3	Load 1	Pool 2	Load 4	PCR Control (+)
Std 4	Std 4	Std 4	Pool 1	Pool 2	Load 4	
Std 5	Std 5	Std 5	Pool 1	Load 3	Load 4	
Std 6	Std 6	Std 6	Pool 1	Load 3	Pool 4	NTC
Std 7	Std 7	Std 7	Load 2	Load 3	Pool 4	NTC
Std 8	Std 8	Std 8	Load 2	Pool 3	Pool 4	NTC

REFERENCES

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WARRANTY

There are no warranties pertaining to its overall performance or shelf-life. In no instance shall MockV Solutions be liable for any proximate or incidental damages in connection with the product.

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