# *MSD® 96-Well MULTI-ARRAY Phospho-VASP Assay*

*The following assay protocol has been optimized for quantifying phosphorylated VASP in whole cell lysates.*

## Materials Included

| | Storage |
|---|---|
| ❑ MULTI-SPOT® 96-Well, 4-Spot Phospho-VASP Plate(s) | 2–8ºC |
| ❑ SULFO-TAG™ Anti-Total VASP Antibody (50X)[1] | 2–8ºC |
| ❑ Tris Wash Buffer (10X) | 2–8ºC |
| ❑ Tris Lysis Buffer (1X) | 2–8ºC |
| ❑ Protease Inhibitor Solution (100X) | 2–8ºC |
| ❑ Phosphatase Inhibitor I (100X) | 2–8ºC |
| ❑ Phosphatase Inhibitor II (100X) | 2–8ºC |
| ❑ Blocker D-R (10%)[2] | ≤-10ºC |
| ❑ Blocker A | RT |
| ❑ Read Buffer T (4X) | RT |

**Note:** A spot map identifying the location of the assay can be found on the plate packaging. This information will be needed for data analysis.

## *Safety*

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional safety information is available in the product safety data sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

[2] Blocker D-R can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of blocker D-R can be stored at 2–8°C for up to 1 month.

## Other Materials & Equipment (not supplied)

Notes:

- ❑ Deionized water for diluting concentrated buffers
- ❑ 500 mL bottle
- ❑ 50 mL tubes
- ❑ 15 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of samples (if desired)
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 µL and 150 µL into a 96-well micro plate

## Protocol at a Glance

*Read the entire detailed instructions before beginning work.*

1. Add blocking solution, incubate 1 hour, wash.
2. Add samples or lysate, incubate 1 hour, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer T and analyze plate.

The full protocol that follows describes the most conservative approach to achieving highly sensitive results using MSD technology to quantify phosphoproteins. The protocol can be completed in approximately 3 to 3 1/2 hours if reagent for each step is prepared during the preceding incubation. Alternatively, all reagents with the exception of diluted samples can be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubation and wash steps and increase throughput.

## Detailed Instructions

**Prepare a stock of 1X Tris Wash Buffer.** 1X Tris Wash Buffer is used throughout the assay to dilute other reagents and wash plates. Approximately 350 mL per plate is required—more if using an automated plate washer.

*A larger amount of Tris Wash Buffer may be prepared and stored at room temperature.*

In a 500 mL bottle, combine:

- ❑ 35 mL 10X Tris Wash Buffer
- ❑ 315 mL deionized water

**Prepare Blocker A Solution.** You will need 20 mL per plate.

*Solutions containing Blocker A should be kept at 2–8ºC and discarded after 5 weeks.*

In a 50 mL tube, combine:

- ❑ 20 mL 1X Tris Wash Buffer
- ❑ 600 mg Blocker A (3% w/v)

**Prepare Antibody Dilution Buffer.**
You will need 3 mL per plate.

In a 15 mL tube, combine:

- ❑ 1 mL Blocker A solution
- ❑ 1.97 mL 1X Tris Wash Buffer
- ❑ 30 µL 10% Blocker D-R

## STEP 1

**Begin** with the Phospho-VASP plate. No pre-treatment is necessary.

**Add** 150 µL/well of Blocker A solution.

*You may also block plates overnight at 2–8ºC.*

**Incubate** with shaking at room temperature for 1 hour. During this time, prepare complete lysis buffer and dilute cell lysates.

*Prepare complete lysis buffer immediately prior to use.*

**Prepare complete lysis buffer.** All of the reagents must be at room temperature before mixing.

To 9.7 mL of Tris Lysis Buffer, add:

- ❑ 100 µL Protease Inhibitor Solution (100X stock)
- ❑ 100 µL Phosphatase Inhibitor I (100X stock)
- ❑ 100 µL Phosphatase Inhibitor II (100X stock)

*Complete lysis buffer should be ice cold before use.*

Keep complete lysis buffer on ice until use.

**Prepare positive and negative samples.**

1) Thaw cell lysate samples on ice, and dilute immediately before use. Keep on ice during all manipulations, and discard all unused, thawed material.
2) Dilute positive and negative cell lysates in complete lysis buffer to a final concentration of 0.8 µg/µL. This will deliver 20 µg/well in 25 µL. A dilution series may also be prepared as needed.

## STEP 2

**Wash** plate(s) three times with at least 150 µL/well of 1X Tris Wash Buffer.

**Dispense** 25 µL/well of the diluted lysates prepared during Step 1 incubation.

**Incubate** with shaking for 1 hour at room temperature. During this time, prepare detection antibody solution.

*Shaking the plate accelerates analyte capture.*

**Prepare Detection Antibody Solution.** You will need 3.0 mL per plate at a 1X final concentration.

In a 15 mL tube, combine:

- ❑ 2.94 mL cold antibody dilution buffer
- ❑ 60 µL 50X SULFO-TAG Anti-Total VASP Antibody

## STEP 3

**Wash** plate(s) three times with at least 150 µL/well of 1X Tris Wash Buffer.

**Add** 25 µL/well of detection antibody solution.

**Incubate** with shaking at room temperature for 1 hour. During this time, prepare Read Buffer T.

*Diluted read buffer may be kept in a tightly sealed container at room temperature for later use.*

**Prepare Read Buffer T.** You will need 20 mL per plate at a final 1X concentration.

In a 50 mL tube, combine:

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

*Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.*

## STEP 4

**Wash** plate(s) three times with at least 150 µL/well of 1X Tris Wash Buffer.

*Read plate(s) immediately after adding read buffer.*

**Add** 150 µL/well of diluted Read Buffer T.

**Analyze** with MSD instrument.