

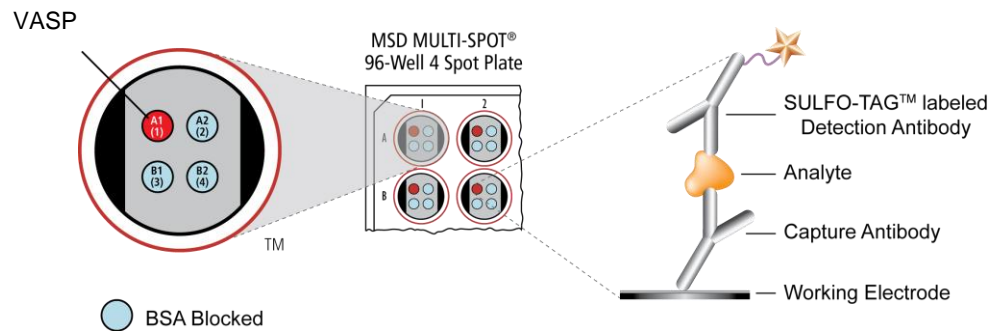
MSD[®] 96-Well MULTI-ARRAY Phospho-VASP Assay

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

Storage

MSD Materials

<input type="checkbox"/> MULTI-SPOT [®] 96-well 4 Spot Phospho-VASP plate(s)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti-Total VASP Antibody (50X)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> Tris Lysis Buffer (1X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor I (100X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor II (100X)	2-8°C
<input type="checkbox"/> Protease Inhibitor Solution (50X)	≤-10°C
<input type="checkbox"/> Blocker D-R (10%)	≤-10°C ¹
<input type="checkbox"/> Blocker A	RT
<input type="checkbox"/> Read Buffer T (4X)	RT



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¹ 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.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Notes:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer
- One 250 mL bottle
- Two 50 mL tube
- One 15 mL tube
- Adhesive plate seals
- Microtiter plate shaker
- Various microcentrifuge tubes for making serial dilutions of lysates (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 μ L and 150 μ L into a 96-well micro plate

Read the entire detailed instructions before beginning work.

Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify phosphoproteins. The protocol takes approximately 3 to 3 1/2 hours to complete if each reagent is prepared during the preceding incubation. All reagents with the exception of diluted lysates can also 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 to increase throughput.

1. Add Blocking Solution, 1 hour, wash.
2. Add samples or lysate, incubate 1 hour, wash.
3. Add Detection Antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

- a) 1X Tris Wash Buffer will be used throughout the assay to make other reagents as well as wash plates. Approximately 250 mL per plate is required – more if using an automatic plate washer.
- b) In a 250 mL bottle combine:
 - 25 mL 10X Tris Wash Buffer
 - 225 mL deionized water

A larger amount of Wash Buffer may be prepared and stored at room temperature for later use.

Prepare Blocking Solution-A:

- a) Prepare 25 mL per plate.
- b) In a 50 mL tube combine:
 - 25 mL 1X Tris Wash Buffer
 - 750 mg Blocker A (30 mg/mL or 3%)

Solutions containing Blocker A should be kept at 4°C and discarded after 14 days.

Prepare Antibody Dilution Buffer:

- a) Prepare 3 mL per plate.
- b) In a 15 mL tube combine (estimated for one plate):
 - 1 mL Blocking Solution-A
 - 1.97 mL 1X Tris Wash Solution
 - 30 μ L 10% Blocker D-R



Begin with a MULTI-SPOT Phospho-VASP Plate.
No pre-treatment is necessary.

STEP 1

Add 150 μL /well of Blocking Solution-A.
Incubate with shaking at room temperature for 1 hour. Prepare Complete Tris Lysis Buffer, and prepare samples or dilute cell lysates during this time.

Prepare Complete Lysis Buffer:

- a) Prepare 10 mL Complete Lysis Buffer. To 10 mL of Tris Lysis Buffer, add the following:
 - 200 μL Protease Inhibitor Solution (50X stock)
 - 100 μL Phosphatase Inhibitor I (100X stock)
 - 100 μL Phosphatase Inhibitor II (100X stock)
- b) Keep Complete Lysis Buffer on ice until use.

Prepare samples or positive and negative cell lysates:

(Note: Recommendations for cell lysate handling are provided, however, the suggested concentrations listed below may need to be adjusted depending upon specific samples tested.)

- a) Thaw cell lysate samples on ice and dilute immediately before use. Keep on ice during all manipulations and discard all remaining thawed unused material.
- b) Dilute cell lysate in Complete Tris Lysis Buffer to a final concentration of 0.8 $\mu\text{g}/\mu\text{L}$. This will deliver 20 μg /well in 25 μL . A dilution series may also be prepared if desired.

Wash plates four times with Wash Buffer.

STEP 2

Dispense 25 μL /well of samples or diluted lysates.
Incubate with shaking at room temperature for 1 hour. Prepare SULFO-TAG Anti-Total VASP Antibody solution during this time.

Prepare Detection Antibody Solution:

- a) Prepare 3.0 mL per plate.
- b) In a 15 mL tube combine:
 - a. 2.94 mL cold Antibody Dilution Buffer
 - b. 60 μL 50X Anti-Total VASP Antibody (Final concentration: 1X)

Wash plates four times with Wash Buffer.

STEP 3

Add 25 μL /well of Detection Antibody Solution.
Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

Dilute Read Buffer:

- a) Approximately 20 mL per plate is required.
- b) Dilute 4X Read Buffer T to 1X with deionized water.

Wash plates four times with Wash Buffer.

STEP 5

Add 150 μL /well of diluted Read Buffer T.
Analyze with SECTOR[®] Imager plate reader.

Notes:

Plates may also be blocked overnight at 4°C.

Complete Tris Lysis Buffer should be made each day of experimentation.

The Complete Lysis Buffer should be ice cold before use.

Shaking a 96-well MULTI-ARRAY[®] or MULTI-SPOT plate accelerates capture at the working electrode.

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

Bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

Plates can be imaged immediately following the addition of Read Buffer.

