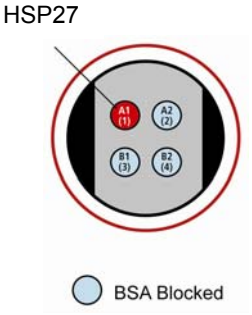
MSD® 384-Well MULTI-ARRAY® Phospho-HSP27 (Ser 15) Assay

The following assay protocol has been optimized for quantifying phosphorylated HSP27 at serine 15 in whole cell lysate.

MSD Materials

	Storage
❑ Read Buffer T(with surfactant), 4X	RT
❑ Blocker A	RT
❑ Blocker B	RT
❑ MULTI-SPOT® 384-well 4 Spot Phospho-HSP27 (Ser 15) Plate(s)	2-8ºC
❑ SULFO-TAG™ Anti-Total HSP27 Antibody (50X)	2-8ºC
❑ Tris Wash Buffer (10X)	2-8ºC
❑ Tris Lysis Buffer (1X)	2-8ºC
❑ Phosphatase Inhibitor I (100X)	2-8ºC
❑ Phosphatase Inhibitor II (100X)	2-8ºC
❑ Protease Inhibitor Solution (50X)	≤-10ºC



The SECTOR® Imager data file will identify spots according to their well location, not by the coated capture antibody name.

Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Wash Buffer and Read Buffer
- ❑ One 1 L 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 384-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 10 µL and 35 µL into a 384-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 5 to 5 ½ 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, incubate 1 hour, wash.
2. Add sample or lysate, incubate 2 hours, wash.
3. Add Detection Antibody, incubate 2 hours, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

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

Prepare a stock of 1X Tris Wash Buffer:

a) The stock of 1X Tris Wash Buffer will be used throughout the assay to make other reagents as well as wash plates. Approximately 1 L per plate is required– more if using an automatic plate washer.

b) In a 1 L bottle combine:
   - ❑ 100 mL 10X Tris Wash Buffer
   - ❑ 900 mL deionized water

Prepare Blocking Solution-A:

a) For blocking and the preparation of additional solutions, 20 mL per plate is required.
b) In a 50 mL tube combine:
   - ❑ 20 mL 1X Tris Wash Buffer
   - ❑ 600 mg Blocker A (30 mg/mL or 3%)

Notes:

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

Prepare Antibody Dilution Buffer:

a) Prepare 8 mL per plate.
b) In a 15 mL tube combine (estimated for one plate):
   - ❑ 2.67 mL Blocking Solution-A
   - ❑ 5.33 mL 1X Tris Wash Solution

Begin with a MULTI-SPOT 384-well 4 Spot Phospho-HSP27 Plate. No pre-treatment is necessary.

STEP 1 Add 35 µL/well of Blocking Solution-A.

Incubate at room temperature with shaking for 1 hour. Prepare Complete Tris Lysis buffer, and prepare samples or dilute cell lysates during this time.

Plates may also be blocked overnight at 4ºC.

Prepare Complete Tris Lysis Buffer:

a) 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 Tris Lysis Buffer on ice until use.

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

The Complete Tris Lysis Buffer should be ice cold before use.

Prepare 5% Blocking Solution-B:

a) Prepare 1 mL 5% Blocking Solution-B. Combine the following:
   - ❑ 50 mg Blocker B
   - ❑ 1 mL Complete Lysis Buffer
b) Mix well to completely dissolve Blocker B.
c) Keep 5% Blocking Solution-B 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.)

Notes:

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 lysates and 5% Blocking Solution-B together in Complete Lysis Buffer to prepare a sample containing 0.5 µg/µL of lysate (5 µg/well in 10 µL) and 0.5% Blocking Solution-B.
c) *For example*, to prepare 200 µL of 0.5 µg/µL lysate-0.5% Blocking Solution-B, combine the following: 20 µL 5% Blocking Solution-B, 50 µL 2 µg/µL lysate and 130 µL Complete Lysis Buffer.
d) Additional dilutions may be prepared from the 0.5 µg/µL lysate sample if desired. Use a stock of Complete Lysis Buffer-0.5% Blocking Solution-B (dilute 5% Blocking Solution-B 1:10 in Complete Lysis Buffer) for any further lysate dilutions.

Wash plates three times with Wash Buffer.

STEP 2

Dispense 10 µL/well of samples or diluted lysates.

Incubate with shaking at room temperature for 2 hours. Prepare Detection Antibody solution during this time.

Shaking a 384-well MSD MULTI-ARRAY or MULTI-SPOT plate accelerates capture at the working electrode.

Prepare Detection Antibody:

a) Prepare 8.0 mL per plate.
b) In a 15 mL tube combine:
    a. 7.84 mL cold Antibody Dilution Buffer
    b. 160 µL 50X Anti-Total HSP27 Antibody (Final concentration: 1X)

Wash plates four times with Wash Buffer.

STEP 3

Add 10 µL/well of Detection Antibody.

Incubate with shaking at room temperature for 2 hours. Prepare Read Buffer during this time.

Dilute Read Buffer:

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

In a 50 mL tube, combine (per plate):
- ☐ 5 mL 4X Read Buffer T
- ☐ 15 mL deionized water

Wash plates four times with Wash Buffer.

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. Most biological interactions tolerate incubation in Read Buffer however each unique assay should be tested for stability in read buffer before being left to sit for extended periods.

STEP 4

Add 35 µL/well of diluted Read Buffer T (with surfactant).

Analyze with SECTOR Imager.