MSD® 384-Well MULTI-ARRAY® Phospho-p53 Assay

The following assay protocol has been optimized for quantifying phosphorylated p53 in whole cell lysate.

MSD Materials

	Storage
❑ Read Buffer T (with surfactant), 4X	RT
❑ Blocker A	RT
❑ Blocker B	RT
❑ MULTI-ARRAY® 384-well Custom plates	2-8ºC
❑ SULFO-TAG™ detection antibody	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
❑ Blocker D-R (10%)	≤-10ºC[1]

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 20 µL and 350 µL into a 384-well micro plate

[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.

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 can be completed in approximately 5 ½ hours or overnight 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 samples or lysate, incubate 2 hours at room temperature or overnight at 4°C, wash.
3. Add detection antibody, incubate 2 hours, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

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) Prepare 20 mL per plate.

b) In a 50 mL tube combine:
- ❑ 20 mL 1X Tris Wash Buffer
- ❑ 600 mg Blocker A (30 mg/mL or 3%)

Prepare Antibody Dilution Buffer:

a) Prepare 9 mL per plate.

b) In a 15 mL tube combine:
- ❑ 3 mL Blocking Solution-A
- ❑ 5.91 mL 1X Tris Wash Buffer
- ❑ 90 µL 10% Blocker D-R

Notes:

Read the entire detailed instructions before beginning work.

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

Solutions containing Blocker A should be dissolved 10-30 min, kept at 4ºC and discarded after 14 days.

Save the plate packaging or copy the diagram of the capture antibody array into your notebook. Data will be labeled according to the location of each spot, not the actual name of the coating.

Begin with a MULTI-ARRAY 384-well Custom Plate.
No pre-treatment is necessary.

STEP 1

Add 35 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 5% Blocking Solution-B:

a) Prepare 1 mL 5% Blocking Solution-B.
   - ❑ 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.)

a) Slowly thaw lysates on ice.

b) Dilute lysates and 5% Blocking Solution-B together in Complete Lysis Buffer to prepare a sample containing 0.5µg/µL of lysate (10 µg/well in 25 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 mg/mL 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.

e) Keep all diluted cell lysate on ice until use.

Wash plates four times with Wash Buffer.

Notes:

Plates may also be blocked overnight at 4ºC.

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

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

Notes:

STEP 2

Dispense 20 µL/well of samples or diluted lysates.

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

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

Prepare Detection Antibody Cocktail:

a) Dilute SULFO-TAG Detection Antibody to a final concentration of 10 nM.
b) Use cold Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 9 mL per plate.

Wash plates four times with Wash Buffer.

STEP 3

Add 20 µL/well of Detection Antibody Cocktail.

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

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

Dilute Read Buffer:

a) Approximately 20 mL per plate is required.
b) Dilute 4X Read Buffer T (with surfactant) to 2X with 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.

STEP 4

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

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.

Analyze with SECTOR® Imager.