MSD 384-Well MULTI-ARRAY® Custom Phospho-p38 Assay

The following assay protocol has been optimized for analysis of phosphorylated p38 in human, mouse, rat, and non-human primate whole cell lysate.

MSD® Materials

	Storage
❑ Custom 384-well Phospho-p38 Plate(s)	2–8ºC
❑ SULFO-TAG™ Anti-p38 Antibody (50X)[1]	2–8ºC
❑ Tris Wash Buffer (10X)	2–8ºC
❑ Tris Lysis Buffer	2–8ºC
❑ Phosphatase Inhibitor I (100X)	2-8ºC
❑ Phosphatase Inhibitor II (100X)	2-8ºC
❑ Protease Inhibitor Solution (100X)	2-8ºC
❑ Blocker A	RT
❑ Read Buffer T (4X)	RT

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting concentrated buffers
- ❑ 1 L bottle
- ❑ 50 mL tubes
- ❑ 15 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of supernatants (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 40 µL into a 384-well micro plate

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

Note:

- A spot map identifying the location of each assay can be found on the plate packaging. This information will be needed for data analysis.
- 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 material safety data sheet, which can be obtained from MSD Customer Service

Protocol at a Glance

Notes:

1. Add blocking solution, incubate 1 hour, wash.
2. Add lysate, incubate 2 hours. Wash.
3. Add detection antibody, incubate 2 hours, 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 5 to 5 ½ hours.

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

Detailed Instructions

Read the entire detailed instructions before beginning work.

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 1 L per plate is required—more if using an automatic plate washer.

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

In a 1 L bottle, combine:

- ❑ 100 mL 10X Tris Wash Buffer
- ❑ 900 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 14 days.

In a 50 mL tube, combine:

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

Prepare Antibody Dilution Buffer. You will need 8 mL per plate.

In a 15 mL tube, combine:

- ❑ 2.67 mL Blocker A solution
- ❑ 5.33 mL 1X Tris Wash Buffer

Set aside on ice.

Begin with a 384-well custom Phospho-p38 plate. No pre-treatment is necessary.

Notes:

STEP 1

Add 40 µL/well of Blocker A solution.

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

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

Prepare complete lysis buffer. All of the reagents must be at room temperature before mixing. You will need 10 mL per plate.

Prepare complete lysis buffer immediately prior to use.

In a 15 mL tube, combine:

- ❑ 9.7 mL of Tris Lysis Buffer
- ❑ 100 µL Phosphatase Inhibitor I (100X stock)
- ❑ 100 µL Phosphatase Inhibitor II (100X stock)
- ❑ 100 µL Protease Inhibitor Solution (100X stock)

Place complete lysis buffer on ice.

Prepare positive and negative samples

1) Thaw 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 lysates in ice-cold complete lysis buffer to a final concentration of 0.5 µg/µL. This will deliver 5 µg/well of lysate in 10 µL of buffer. A dilution series may also be prepared if desired.

STEP 2

Wash plate(s) four times with 70 µL/well of 1X Tris Wash Buffer.

Dispense 10 µL/well of the samples prepared during Step 1 incubation.

Shaking the plate accelerates analyte capture.

Incubate with shaking for 2 hours at room temperature. During this time, prepare detection antibody solution.

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Prepare Detection Antibody Solution. You will need 8.0 mL per plate at a 1X final concentration.

In a 15 mL tube, combine:

- ❑ 7.84 mL cold antibody dilution buffer
- ❑ 160 µL 50X Anti-p38 Antibody

STEP 3

Wash plate(s) four times with 70 µL/well of 1X Tris Wash Buffer.

Add 10 µL/well of detection antibody solution.

Incubate with shaking at room temperature for 2 hours. During this time, prepare Read Buffer T.

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

Notes:

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

STEP 4

Wash plate(s) four times with 70 µL/well of 1X Tris Wash Buffer.

Add 40 µL/well of diluted Read Buffer T.

Analyze with MSD Imager.

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

Read plate(s) immediately after adding read buffer.