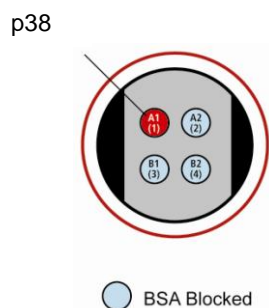


MSD 384-Well MULTI-ARRAY Phospho-p38 Assay

Storage

MSD Materials

<input type="checkbox"/> Read Buffer T (with surfactant), 4X	RT
<input type="checkbox"/> Blocker A	RT
<input type="checkbox"/> Blocker B	RT
<input type="checkbox"/> MULTI-SPOT [®] 384-well 4 Spot Phospho-p38 plate(s)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti-Total p38 Antibody (50X)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> Diluent 20	≤-10°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



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

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



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 20 μ L and 35 μ L 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 can be completed in approximately 5 to 5 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, incubate 1 hour, wash
2. Add lysate, incubate 2 hours, 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

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



Notes:***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%)

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

Prepare Antibody Dilution Buffer:

- a) Approximately 24 mL per plate is required.
- b) In a 50 mL tube combine:
 - 24 mL Diluent 20
 - 120 mg Blocker B (5 mg/mL or 0.5%)

Set aside on ice.

Prepare Complete Low Salt Lysis Buffer:

- a) Bring 10 mL of Diluent 20, add the following:
 - 100 µL Phosphatase Inhibitor I (100X stock)
 - 100 µL Phosphatase Inhibitor II (100X stock)
 - 200 µL Protease Inhibitor Solution (50X)

Keep Complete Low Salt Lysis Buffer on ice until use.

Plates may also be blocked overnight at 4°C.

Prepare 5% Blocking Solution-B:

- a) Combine (per plate):
 - 1 mL Complete Low Salt Lysis Buffer
 - 50 mg Blocker B (5 mg/mL or 0.5%)

Mix well. The 5% Blocking Solution-B should be ice cold before use.

Complete p38 Lysate Dilution Buffer should be made each day of experimentation.

Begin with a MULTI-SPOT 384-well Phospho-p38 Plate.
No pre-treatment is necessary.

The Complete p38 Lysate Dilution Buffer should be ice cold before use.

STEP 1

Add 35 µL/well of Blocking Solution-A.

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

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 p38 positive and negative cell lysates and 5% Blocking Solution-B in Complete Low Salt Lysis Buffer to a final concentration of 0.5 µg/µL. This will deliver 5 µg/well in 10 µL



and 0.5% Blocking Solution-B. A dilution series may also be prepared if desired.

- c) For example, to prepare 50 μL of 0.5 $\mu\text{g}/\mu\text{L}$ lysate-0.5% Blocking Solution-B, combine the following: 5 μL 5% Blocking Solution-B, 12.5 μL 2 mg/mL lysate and 32.5 μL Complete Low Salt Lysis Buffer.
- d) A dilution series may also be prepared if desired. Use a stock of Complete Low Salt Lysis Buffer-0.5% Blocking Solution-B (dilute 5% Blocking Solution-B 1:10 in Complete Low Salt Lysis Buffer) for any subsequent lysate dilutions.

Wash plates four times with Wash Buffer.

STEP 2

Dispense 10 μL /well of samples or diluted lysates.

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

Prepare Detection Antibody:

In a 15 mL tube, combine (per plate):

- a. 7.84 mL cold Antibody Dilution Buffer
- b. 160 μL 50X Anti-Total p38 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:

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.

STEP 4

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

Analyze with SECTOR Imager.

Notes:

Shaking a 384-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. 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.

