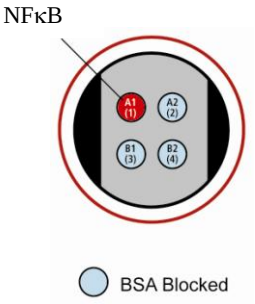
MSD® 384-Well MULTI-ARRAY® Phospho-NFκB (Ser536) Assay

The following assay protocol has been optimized for quantifying phosphorylated NFκB (Ser536) in whole cell lysate.

Storage

Materials Included

❑ Read Buffer T (with surfactant), 4X	RT
❑ Blocker A	RT
❑ MULTI-SPOT® 384-well 4 Spot Phospho-NFκB (Ser536) Plate(s)	2-8ºC
❑ SULFO-TAG™ Anti-Total NFκB 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 tubes
- ❑ 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, 20, 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 can be completed in approximately 5 to 5 1/2 hours 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 at room temperature, wash.
3. Add Detection Antibody, incubate 2 hours, wash.
4. Add Read Buffer and analyze plate.

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

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

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 (3%)

Solutions containing Blocker A should be dissolved 10-30 min, 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:
   - ❑ 2.67 mL Blocking Solution-A
   - ❑ 5.33 mL 1X Tris Wash Buffer

Begin with a MULTI-SPOT 384-well 4 Spot Phospho-NFκB (Ser536) Plate. No pre-treatment is necessary.

STEP 1

Add 35 µL/well of Blocking Solution-A.

Plates may also be blocked overnight at 4ºC.

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

Prepare Complete Tris Lysis Buffer:

a) To 10 mL of Tris Lysis Buffer, add the following:
   - ❑ 100 µL Phosphatase Inhibitor I (100X stock)
   - ❑ 100 µL Phosphatase Inhibitor II (100X stock)
   - ❑ 200 µL Protease Inhibitor Soution (50X 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 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 positive and negative cell lysates in Complete Tris Lysis Buffer to a final concentration of 0.5 µg/µL. This will deliver 5 µg/well in 10 µL. A dilution series may also be prepared if desired.

Wash plates three times with Wash Buffer.

Notes:

STEP 2

Dispense 10 µL/well of diluted lysates.

Incubate with shaking for 2 hours at room temperature. 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 Solution:

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 NFκB Antibody (Final concentration: 1X)

Wash plates three times with Wash Buffer.

STEP 3

Add 10 µL/well of Detection Antibody Solution.

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

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

Wash plates four times with Wash Buffer.

STEP 4

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

Analyze with SECTOR Imager.

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.