

MSD[®] Multiplex Apoptosis Panel: Phospho p53 (pSer15), Total p53, Cleaved PARP and Cleaved Caspase-3
MULTI-SPOT[®] 4, 96-well Plate

Storage

Materials Included

<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 4, 96-well Apoptosis Panel Plate(s)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] p53 Detection Antibody (50X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] PARP Detection Antibody (50X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Caspase-3 Detection Antibody (50X)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> Tris Lysis Buffer (1X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor I (100X)	2-8°C
<input type="checkbox"/> Phosphatase Inhibitor II (100X)	2-8°C
<input type="checkbox"/> Blocker D-R (10%)	≤-10°C
<input type="checkbox"/> Protease Inhibitor Solution (50X)	≤-10°C

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer
- One 250 mL bottle
- Two 50 mL tube
- One 15 mL tube
- Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- Adhesive plate seals
- Microtiter plate shaker
- Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 µL and 150 µL into a 96-well micro plate



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 3 to 3 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 1 hour at room temperature, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

- a) 1X Tris Wash Buffer will be used throughout the assay to make other reagents as well as wash plates. Approximately 250 mL per plate is required – more if using an automatic plate washer.
- b) In a 250 mL bottle combine:
 - 25 mL 10X Tris Wash Buffer
 - 225 mL deionized water

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.

Prepare Blocking Solution-A:

- a) Prepare 20 mL per plate.
- b) In a 50 mL tube combine:
 - 20 mL 1X Tris Wash Buffer
 - Blocker A 600mg (3%)

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

Prepare Antibody Dilution Buffer:

- a) Prepare 3 mL per plate.
- b) In a 15 mL tube combine:
 - 1 mL Blocking Solution-A
 - 1.97 mL 1X Tris Wash Buffer
 - 30 µL 10% Blocker D-R



Notes:

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.

Plates may also be blocked overnight at 4°C.

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

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

Begin with a MULTI-SPOT Apoptosis Panel Plate. No pre-treatment is necessary.

STEP 1

Add 150 µL/well of Blocking Solution.

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) It is important that all of the reagents (including the Tris Lysis Buffer) are room temperature before mixing. 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 Solution (50X stock)
- b) Keep Complete Tris Lysis Buffer on ice until use.

Prepare 5% Blocking Solution-B:

- a) Prepare 1mL 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.)

- 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) Assays that use Blocker B:
 1. Dilute lysates and 5% Blocking Solution-B together in Complete Lysis Buffer to prepare a sample containing 0.8 µg/µL of lysate (20 µg/well in 25 µL) and 0.5% Blocking Solution-B.
 2. For example, to prepare 200 µL of 0.8 µg/µL lysate-0.5% Blocking Solution-B, combine the following: 20 µL 5% Blocking Solution-B, 80 µL 2 µg/µL lysate and 100 µL Complete Lysis Buffer.
 3. Additional dilutions may be prepared from the 0.8 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.



Notes:

STEP 2 Dispense 25 μ L/well of diluted lysates.

Incubate with shaking for 1 hour at room temperature. Prepare Detection Antibody Cocktail during this time.

Prepare Detection Antibody Cocktail:

- Dilute SULFO-TAG Detection Antibodies in a common volume to a final concentration of 1X for p53, PARP, and Caspase-3.
- Use cold Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 3 mL per plate.

Wash plates three times with Wash Buffer.

Shaking a 96-well MSD MULTI-ARRAY[®] or MULTI-SPOT plate accelerates capture at the working electrode.

For example, if each antibody is supplied at 50X, you would add 60 μ L of EACH antibody to a single tube containing 3 mL of Antibody Dilution Buffer.

STEP 3 Add 25 μ L/well of Detection Antibody Cocktail.

Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

Dilute Read Buffer:

- Approximately 20 mL per plate is required.
- Dilute 4X Read Buffer T (with surfactant) to 1X with deionized water.

If certain signals are judged to be too great relative to others in the array, the final concentration of the corresponding detection antibody can be reduced in an effort to equilibrate the signals.

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

STEP 4 Add 150 μ L/well of diluted Read Buffer T (with surfactant).

Analyze with SECTOR[®] Imager plate reader.

Plates can be imaged immediately following the addition of read buffer.

Note that bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

