

MSD[®] 96-Well MULTI-ARRAY[®] Ubiquitinated p53

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

	Storage
<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 96-well 4 Spot p53 Plate(s)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti-Ubiquitinated Protein Detection Antibody	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"/> Blocker D-R (10%)	≤-10°C ¹
<input type="checkbox"/> Blocker D-M (2%)	≤-10°C ¹
<input type="checkbox"/> Protease Inhibitor Solution (50X)	≤-10°C

II. Other Materials & Equipment (not supplied)

- EDTA, 0.5 M
- Deionized water for diluting Wash Buffer and Read Buffer
- One 250 mL 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 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

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¹ Blockers D-M and D-R can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of blockers D-M and D-R can be stored at 2-8°C for up to 1 month.

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



III. Preparation and General Notes

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

Prepare Blocking Solution B:

- a) Combine:
 - 1 g Blocker B (dry powder)
 - 20 mL 1X Tris Wash Buffer
- b) Mix until all materials are completely dissolved. Keep Complete Tris Lysis Buffer on ice until use.

Prepare Complete Lysate Dilution Buffer:

- a) To 10 mL of 1X Tris Lysis Buffer, add the following:
 - 200 μ L Protease Inhibitor Solution (50X stock)
 - 400 μ L 0.5 M EDTA
 - 500 mg Blocker B
- b) Mix until all materials are completely dissolved. Keep Complete Tris Lysis Buffer on ice until use.

Prepare cell lysate dilutions:

- 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 cell lysates in Complete Lysate Dilution Buffer. Typical dilutions range from 0.08-5 μ g/well.

Prepare Antibody Dilution Buffer:

Combine (per plate):

- 30 mg Blocker A
- 2.82 mL Tris Wash Buffer
- 30 μ L 10% Blocker D-R
- 150 μ L 2% Blocker D-M

Prepare SULFO-TAG labeled Detection Antibody Solution:

- a) Dilute SULFO-TAG anti-ubiquitinated protein antibody to 0.75 μ g/mL (5 nM) in Antibody Dilution Buffer.
- b) Approximately 3 mL per plate is required.

Prepare Read Buffer:

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

Notes:

Read the entire detailed instructions before beginning work.

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

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

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

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

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



Notes:

IV. Detailed Instructions

MSD Assay for Ubiquitinated p53

- 1) Add 150 μ L/well of Blocking Solution B and incubate at room temperature for 1 hour. Prepare diluted lysates during this time.
- 2) Wash plates four times with 1X Tris Wash Buffer.
- 3) Dispense 25 μ L/well of diluted lysates. Incubate with shaking at room temperature for 3 hours. Prepare Detection Antibody Solution during this time.
- 4) Wash plates four times with 1X Tris Wash Buffer.
- 5) Dispense 25 μ L/well of diluted SULFO-TAG anti-ubiquitinated protein antibody. Incubate with shaking at room temperature for 1 hour.
- 6) Wash plates four times with 1X Tris Wash Buffer.
- 7) Dispense 150 μ L/well of 1X Read Buffer T, with surfactant, and analyze with the SECTOR[®] instrument.

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

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.

V. Additional Notes

Please note: The following lysate preparation protocol is provided for reference purposes.

HCT116 Cell Lysate Preparation

- 1) Prepare Complete Cell Lysis Buffer:
 - 10 mL of 1X Tris Lysis Buffer
 - 200 μ L Protease Inhibitor Solution (50X stock)
 - 100 μ L Phosphatase Inhibitor I (100X stock)
 - 100 μ L Phosphatase Inhibitor II (100X stock)
 - 400 μ L 0.5 M EDTA
 - 100 μ L 2 M NEM (N-ethylmaleimide, make fresh stock in ethanol and add to the Cell Lysis Buffer immediately prior to use)Phosphatase inhibitors are included to allow the same lysate to be used with MSD Phosphoprotein Assays.
- 2) Cells grown in 150 mm dishes should be washed with PBS prior to lysis. All traces of PBS should be carefully removed with a Pasteur pipette.
- 3) Add 1-2 mL per dish of Complete Cell Lysis Buffer, distribute evenly and incubate on ice for 15 minutes. Collect cell lysate with a cell scraper and transfer into an Eppendorf tube. To homogenize lysate, aspirate and dispense with a pipet. Centrifuge for 10 minutes at 16,000xg, 4°C. Aliquot and store supernatant at -80°C.