MSD® MULTI-ARRAY Assay System

Phospho-p53 (Ser15) Assay Whole Cell Lysate Kit

1-Plate Kit	K151DAD-1
5-Plate Kit	K151DAD-2
20-Plate Kit	K151DAD-3

MSD Phosphoprotein Assays

Phospho-p53 (Ser15) Assay
Whole Cell Lysate Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Ordering Information

MSD Customer Service

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MSD Scientific Support

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MSD Advantage

MESO SCALE DISCOVERY'S unique spot patterns are a hallmark of our MULTI-ARRAY® technology which measures multiple biomarkers using next generation electrochemiluminescence detection. In a MULTI-ARRAY assay, specific capture antibodies are coated in arrays in each well of a 96- or a 384-well carbon electrode plate. The detection system uses patented SULFO-TAG™ labels that emit light upon electrochemical stimulation, resulting in assays with low background. MSD SULFO-TAG labels are stable, non-radioactive, and easily conjugated to biological molecules. Electrochemiluminescence is a proximity assay, so only labels near the electrode surface are excited, making non-washed assays possible.

Compared to MSD technology, an ELISA requires larger sample volume, measures only one analyte at a time, and has a more complicated, time-consuming protocol. With an MSD electrochemiluminescence assay, up to ten different biomarkers can be analyzed simultaneously using as little as 10–25 µL of sample. MSD assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. The simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results, reducing workflow without compromising data quality. The result is an increase in productivity and a decrease in cost per analyte.

The MSD product line offers an extensive menu of assay kits for quantifying biomarkers and dissecting cell signaling pathways as well as custom and prototype assays designed to customer specifications. Plates and reagents are available for customers wishing to develop novel assays on the MSD platform.

Introduction

p53 (protein 53) is a transcription factor and tumor suppressor protein with an apparent molecular weight of 53 kDa that plays a critical role in cell cycle regulation, progression, and apoptosis.[1] p53 is composed of an amino terminal transcription activation domain, a proline rich domain critical to the p53 apoptotic functions, a central DNA binding domain, a nuclear localization sequence, and an oligomerization domain.[2] The extent of DNA damage and different types of cellular stress determine the set of downstream targets activated by p53. MDM2 is a potent negative regulator of p53 through its binding and subsequent polyubiquitination of p53, resulting in proteasome dependent degradation.[3] This negative regulation can be relieved both through phosphorylation of p53, resulting in destabilization of the MDM2-p53 interaction,[4] and through phosphorylation and ubiquitination of MDM2.[1]

p53 is the most commonly mutated gene in cancer, and a functional copy of p53 is required to maintain a non-tumorigenic phenotype.[5] When cell repair is possible, p53 activates genes that pause the cell cycle allowing time for DNA repair, but when damage is extensive, p53 activates the BCL-2 family of proteins leading to apoptosis.[6] p53's role as a transcription factor and the negative regulation of the protein by MDM2 mediated polyubiquitination has been extensively researched due to its crucial role in cancer prevention and cell cycle control.

Principle of the Assay

MSD phosphoprotein assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. The Phospho-p53 (Ser15) Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibody for phospho-p53. The user adds the sample and a solution containing the detection antibody—anti-total p53 conjugated with an electrochemiluminescent label, MSD SULFO-TAG —over the course of one or more incubation periods. Analyte in the sample binds to the capture antibody immobilized on the working electrode surface; recruitment of the conjugated detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® Imager for analysis. Inside the SECTOR Imager, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to provide a quantitative measure of phosphorylated p53 present in the sample.

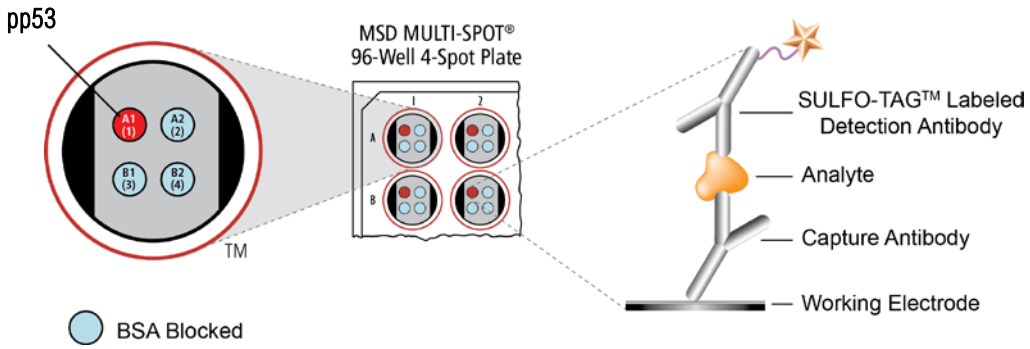


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151DAD-1	K151DAD-2	K151DAD-3
MULTI-SPOT 96-Well 4-Spot Phospho-p53 (Ser15) Plate N451DAB-1	2–8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-Total p53 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	4 vials (375 µL ea)
Tris Lysis Buffer (1X) R60TX-3 (50 mL), R60TX-2 (200 mL)	2–8°C	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)
Tris Wash Buffer (10X) R61TX-2 (200 mL), R61TX-1 (1000 mL)	2–8°C	1 bottle (200 mL)	1 bottle (200 mL)	1 bottle (1000 mL)
Phosphatase Inhibitor I (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	1 vial (2.0 mL)
Phosphatase Inhibitor II (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	1 vial (2.0 mL)
Protease Inhibitor Solution (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	1 vial (2.0 mL)
Blocker D-R[2] (10%)	≤-10°C	1 vial (0.05 mL)	1 vial (0.2 mL)	4 vials (0.2 mL ea)
Blocker A (dry powder) R93BA-4	RT	1 vial (15 g)	1 vial (15 g)	1 vial (15 g)
Blocker B (dry powder) R93BB-1	RT	1 vial (1 g)	1 vial (1 g)	1 vial (2 g)
Read Buffer T (4X) R92TC-3 (50 mL), R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)

Required Materials and Equipment — not supplied

- Deionized water for diluting Tris Wash Buffer (10X) and Read Buffer T (4X)
- 500 mL bottle for reagent preparation
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

[1] Some SULFO-TAG conjugated detection antibodies may be light sensitive, so they should be stored in the dark.
[2] Blocker D-R can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of Blocker D-R can be stored at 2-8°C up to 1 month.

Safety

Safe laboratory practices and personal protective equipment, such as gloves, safety glasses, and lab coats, should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

Reagent Preparation

Prepare Tris Wash Buffer

Dilute the 10X stock of Tris Wash Buffer provided with the MSD kit to 1X as shown below. Tris Wash Buffer (1X) will be used throughout the assay to make additional reagents and wash plates. Approximately 350 mL per plate is required—more if using an automatic plate washer.

For one plate, combine:

- ❑ 35 mL of Tris Wash Buffer (10X)
- ❑ 315 mL deionized water

Excess Tris Wash Buffer may be stored at room temperature in a tightly sealed container for later use.

Prepare Blocking Solution-A

For one plate, combine:

- ❑ 600 mg Blocker A (dry powder)
- ❑ 20 mL 1X Tris Wash Buffer

Prepare Antibody Dilution Buffer

For one plate, combine:

- ❑ 30 µL 10% Blocker D-R
- ❑ 1 mL blocking solution-A
- ❑ 1.97 mL 1X Tris Wash Buffer

Set aside on ice.

Prepare Complete Lysis Buffer

To 10 mL of the Tris Lysis Buffer provided with the MSD kit, add the following supplemental materials to prepare the complete lysis buffer (sufficient for 2-3 plates):

- ❑ 100 µL Protease Inhibitor Solution (100X stock)
- ❑ 100 µL Phosphatase Inhibitor Solution I (100X stock)
- ❑ 100 µL Phosphatase Inhibitor Solution II (100X stock)

The complete lysis buffer should be ice cold before use.

Prepare 5% Blocking Solution-B

For one plate, combine:

- ❑ 50 mg Blocker B (dry powder)
- ❑ 1 mL complete lysis buffer

Mix well. The 5% blocking solution-B should be ice cold before use.

Prepare Detection Antibody Solution

For one plate, combine:

- ❑ 2.94 mL antibody dilution buffer
- ❑ 60 µL 50X SULFO-TAG Anti-Total p53 Antibody (1X final concentration)

Prepare Read Buffer

For one plate, combine:

- ❑ 5 mL Read Buffer T (4X)
- ❑ 15 mL deionized water

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

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

Sample Preparation and Storage

This cell lysis protocol is provided as a reference. Specific cell types or targets may benefit from alternative buffer components or techniques, depending upon the particular research application. Most lysis buffers are compatible with MSD MULTI-SPOT plates, although high concentrations of denaturing detergents (>0.1%) and reducing agents (DTT >1mM) should be avoided. Please contact MSD Scientific Support with any questions regarding lysate preparation options.

All manipulations should be performed on ice. The amount of complete lysis buffer required will vary depending on scale of preparation and type of cells. Larger cells (e.g. NIH3T3, HeLa) should be lysed at concentrations of 1–5 x 10^6 cells per mL of lysis buffer. Smaller cells (e.g. Jurkat) should be lysed at concentrations of 1–5 x 10^7 cells per mL of lysis buffer.

Analysis of proteins in their activated state (i.e. phosphorylated) usually requires stimulation prior to cell lysis. Verification of cell stimulation and sample preparation should be performed prior to using this kit. Phosphate buffered saline (PBS) should be ice-cold prior to use.

Suspension Cells

Pellet cells by centrifugation at 500 x g for 3 minutes at 2–8°C. Discard supernatant and wash the pellet once with cold PBS. Pellet the cells again, discard supernatant, and resuspend in complete lysis buffer at 1–5 x 10^7 cells per mL. Incubate on ice for 30 minutes. (A shorter incubation time of 15 minutes may be adequate for many targets.) Clear cellular debris from the lysate by centrifugation greater than or equal to 10000 x g, at 2–8°C for 10 minutes. Discard the pellet and determine the protein concentration in the lysate using a detergent compatible protein assay such as a bicinchoninic acid (BCA) assay. Unused lysates should be aliquoted, quickly frozen in a dry ice-ethanol bath, and stored at ≤-70°C.

Adherent Cells

All volumes are determined for cells plated in 15 cm dishes. Remove media from the plates and wash cells one time with 5 mL cold PBS. Add 2 mL PBS to the plates, scrape the cells from the surface of the dish, and transfer into 15 mL conical tubes. Pellet the cells by centrifugation at 500 x g for 3 minutes at 2–8°C. Discard supernatant and resuspend cells in 0.5–2 mL of complete lysis buffer per dish. Incubate on ice for 30 minutes. A shorter incubation time of 15 minutes may be adequate for many targets. Clear cellular debris from the lysate by centrifugation greater than or equal to 10 000 x g, at 2–8°C for 10 minutes. Discard the pellet and determine protein concentration in the lysate using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted and quickly frozen in a dry ice-ethanol bath and stored at ≤-70°C.

Refer to Appendix I for cell lysate preparation protocol modifications that accommodate the use of 96-well culture plates.

Assay Protocol

The following protocol describes the most conservative approach to achieving optimal results with the MULTI-ARRAY Phospho-p53 (Ser15) Assay. The entire assay, including plate analysis on the MSD reader, can be completed in 3.5 hours. Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubations and wash steps. Samples may be prepared for testing in the manner outlined in the Sample Preparation and Storage section.

1. **Block Plate and Prepare Samples:**
   a. Add 150 µL of blocking solution-A into each well. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   b. Prepare complete lysis buffer just prior to sample dilution.

      Note: Samples, including cell lysates, may be used neat or after dilution.

      - MSD plates are compatible with most sample matrices. Avoid reagents that will denature the capture antibodies (e.g. high concentrations of reducing agents such as DTT should be avoided; SDS and other ionic detergents should be 0.1% or less in the sample applied to the well).
      - Depending on the stability of the target in the matrix, additional protease and phosphatase inhibitors may be required in the matrix or diluent.
      - If working with purified protein, only a few nanograms per well will generally provide a strong assay signal. Purified recombinant proteins may exhibit differences in both signal and background as compared to native proteins in cell lysates.
      - Keep diluted samples on ice until use.

   c. Prepare positive and negative cell lysates.

      Note: Cell lysates may be purchased separately from MSD.

      - Thaw cell lysate samples on ice, and dilute them immediately before use. Keep on ice during all manipulations, and discard all remaining thawed, unused material.
      - Dilute cell lysate and 5% blocking solution-B together in complete lysis buffer to a final concentration of 0.8 µg/µL. This will deliver 20 µg/well lysate in 25 µL and 0.5% blocking solution-B.

Notes

Read entire protocol prior to beginning the assay.

Solutions containing MSD Blocker A should be stored at 2-8°C and discarded after 14 days.

Complete lysis buffer should be kept ice-cold during all experimental manipulations.

The sensitivity of MSD immunoassays rivals that of ELISAs and Western blots. The amount of sample required for a given assay will depend on the abundance of the analyte in the matrix and the affinities of the antibodies used.

Samples and standards cannot be serially diluted in the MSD plate. Use microcentrifuge tubes or a separate 96-well polypropylene plate to prepare dilutions.

- 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 mg/mL lysate
  - 100 µL complete lysis buffer
- A dilution series may also be prepared 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 subsequent lysate dilutions.

2. **Wash and Add Samples:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 25 µL of sample per well. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Prepare detection antibody solution during this time.

3. **Wash and Add Detection Antibody:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 25 µL of detection antibody solution to each well of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Prepare 1X Read Buffer T during this time.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 150 µL of 1X Read Buffer T to each well of the MSD plate.

   Analyze the plate on the SECTOR Imager:

   a. Double click on the DISCOVERY WORKBENCH® icon on the computer desktop (if not already open).
   b. Click the SECTOR Imager icon in upper left corner of screen (if not already open to plate reading screen).
   c. From the pull down menu, select "Read From Barcode."
   d. If only reading one plate, check "Return Plate to Input Stack." Then check the "Read Plate(s)" checkbox and enter 1.
   e. If reading multiple plates, check the "Read Plate(s)" checkbox and enter number of plates to be read in the text field. For example, if five plates need to be read, enter "5."
   f. Click the "Run" button. The "Run Options" window will be displayed.

Notes

Shaking a 96-well MSD MULTI-ARRAY or MULTI-SPOT plate during an incubation step will typically accelerate capture at the working electrode.
The lysate sample incubation time provided is optimized for the use of MSD cell lysates. Samples from other sources may require a longer incubation.

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

Bubbles introduced during the read buffer addition will interfere with imaging of the plate and produce unreliable data.

Plate should be imaged within 5 minutes following the addition of read buffer. Due to the varying nature of each research application, assay stability should be investigated prior to allowing plates to sit with read buffer for extended periods.

An all-inclusive indelible copy of the data and associated instrument information will be saved on the internal database, regardless of data file export selection. Additional copies of the data can be exported in any layout at a later time using this database. Consult the instrument user manual for more information.

g. If the data from each microplate is to be exported as individual files, select “Separate Files” in the “Export” area of the “Run Options” window. If all data from the entire stack is to be exported to one file, select “Appended File.”

h. In the “Export Format” area, check the box to export default data. If desired, make selections to also export a custom data file.

i. Browse and select the location to which exported data files will be saved. Provide a unique name for the custom file.

j. Click OK to initiate the run.

k. Data will be automatically saved in the software database. Text versions of the requested data files will be exported to the designated folder.

An all-inclusive indelible copy of the data and associated instrument information will be saved on the internal database, regardless of data file export selection. Additional copies of the data can be exported in any layout at a later time using this database. Consult the instrument user manual for more information.

Analysis of Results

The percent phosphoprotein in a sample can be calculated using independent MSD phosphoprotein and total protein singleplex assays or MSD phospho-/total multiplex phosphoprotein assays.

INDEPENDENT ASSAY FORMAT: Anti-Total Singleplex and Anti-Phospho-Singleplex Assays
% Phosphoprotein = (Phospho-signal / Total signal) x 100

MULTIPLEX ASSAY FORMAT: Anti-Total and Anti-Phospho-Assay in the same well
% Phosphoprotein = [(2 x Phospho-signal) / (Phospho-signal + Total signal)] x 100

Note:

1. The above calculation assumes that the capture antibodies on the anti-phospho and anti-total spots have very similar binding affinities.
2. The numerator in the equation contains a distribution factor of 2 based on the assumption that the phosphorylated isoform of the protein binds with a similar affinity to the phospho-specific and total capture antibodies. Given equivalent binding of the phosphorylated isoform to both capture antibodies, half of the phosphorylated species will be captured by the phospho-specific and the other half will be captured by the phosphorylation-independent (total) antibody. Therefore, the phospho-specific signal can be referred to as 2X of the phospho spot.
3. The denominator is "phospho + total" because this represents the total of all the analyte captured on both of the spots.
4. If the % phosphorylation is > 100%, then the distribution factor in the numerator may be adjusted to less than 2X such that the % phosphorylation with the control lysates is 100%.

Example:

Phosphoprotein Assay							
Lysates (µg)	Positive Control Lysate			Negative Control Lysate			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	245	4	1.4	242	6	2.5	
5.0	19235	2342	12.2	461	3	0.6	42

Total Protein Assay							
Lysates (µg)	Positive Control Lysate			Negative Control Lysate			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	561	18	3.2	569	19	3.4	
5.0	7304	1227	16.8	14530	585	4.0	0.5

% Phosphoprotein = [(2 x Phospho signal) / (Phospho signal + Total signal)] x 100

Therefore, % phosphoprotein with 5 µg of positive lysate will be:

[(2 x 19235) / (19235 + 7304)] x 100 = 144% phosphorylation

In this case, the constant in the numerator may be adjusted using the control lysates as follows:

[(1.38 x 19235) / (19235 + 7304)] x 100 = 100% phosphorylation

1.38 should be used as the numerator for further calculations in the same experiment.

Typical Data

Representative results for the MULTI-ARRAY Phospho-p53 (Ser15) Assay are illustrated below. The signal and ratio values provided are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-p53 (Ser15) and total p53 antibodies and are shown for comparison. Growing HT29 cells (negative) were harvested 1 hour after UV irradiation (40 mJ/cm^2) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-p53 antibody on one of the four spatially distinct electrodes per well. Phosphorylated p53 was detected with anti-total p53 antibody conjugated with MSD SULFO-TAG.

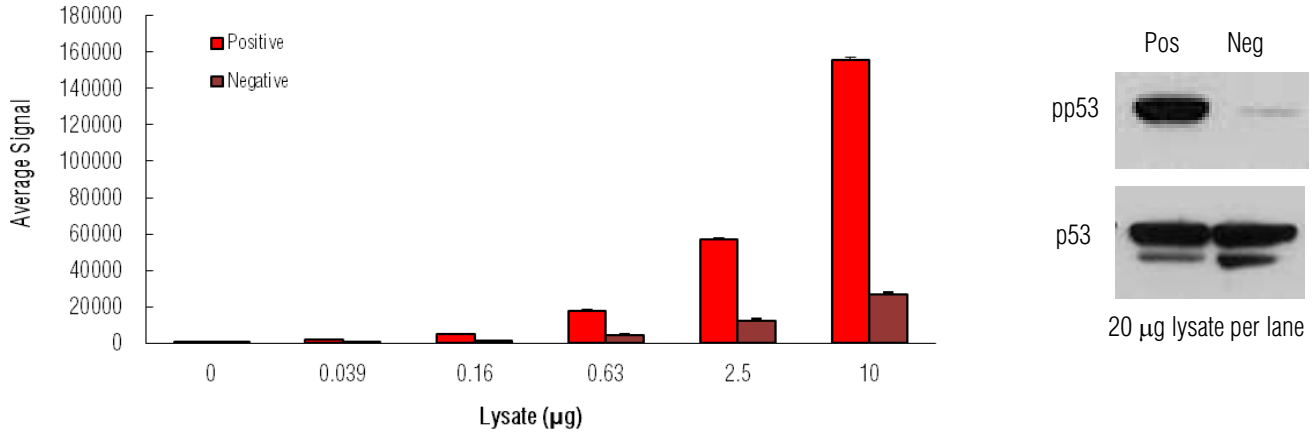


Figure 2: Sample data generated with MULTI-ARRAY Phospho-p53 (Ser15) Assay. Increased signal is observed with the titration of both pp53 positive and negative cell lysates. Signal for pp53 negative cell lysate remains low throughout the titration. The Phospho-p53 (Ser15) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pp53 positive and negative HT29 cell lysates using the MULTI-ARRAY Phospho-p53 (Ser15) Assay is presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	57	8	14.0	41	3	7.3	
0.039	1627	14	0.8	463	1	0.2	3.5
0.16	5241	148	2.8	1383	25	1.8	3.8
0.63	17832	728	4.1	4415	8	0.2	4.0
2.5	56714	1356	2.4	11920	335	2.8	4.8
10	155763	1512	1.0	26580	553	2.1	5.9

Assay Components

The capture and detection antibodies used in this assay are listed below. They cross-react with human whole cell lysates.

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
p53	Mouse Monoclonal	Goat Polyclonal

Limitations of the Procedure

The following points should be noted with the MULTI-ARRAY Phospho-p53 (Ser15) Assay to maximize assay sensitivity and performance.

- A no-wash assay format may be employed; however, lower sensitivity may be observed.
- All buffers containing phosphate should be avoided when detecting phosphoproteins.
- Due to the unstable nature of phosphoproteins, cell lysates should be thawed immediately prior to use, and any remaining thawed material should be discarded.

Companion Products

MULTI-ARRAY Total p53 Assay	
Kit Size	Catalog Numbers
1 plate	K151DBD-1
5 plates	K151DBD-2
20 plates	K151DBD-3
20 plates (Base Kit)	K151DBA-3
MULTI-SPOT Phospho(Ser15)/Total p53 Assay	
Kit Size	Catalog Numbers
1 plate	K15113D-1
5 plates	K15113D-2
20 plates	K15113D-3
20 plates (Base Kit)	K15113A-3
MULTI-SPOT Apoptosis Panel [Cleaved PARP, p53, Phospho-p53, Cleaved Caspase-3]	
Kit Size	Catalog Numbers
1 plate	K15102D-1
5 plates	K15102D-2
20 plates	K15102D-3
20 plates (Base Kit)	K15102A-3

References

1. Inuzuka H, Fukushima H, Shaik S, Wei W. Novel insights into the molecular mechanisms governing Mdm2 ubiquitination and destruction. Oncotarget. 2010 Nov;1(17):685-90.
2. Okorokov AL, Orlova EV. Structural biology of the p53 tumour suppressor. Curr Opin Struct Biol. 2009 Apr;19(2):197-202.
3. Midgley CA, Lane DP. p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. Oncogene. 1997 Sep 4;15(10):1179-89.
4. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000 Mar 10;287(5459):1824-7.
5. Kenzelmann Broz D, Attardi LD. In vivo analysis of p53 tumor suppressor function using genetically engineered mouse models. Carcinogenesis. 2010 Aug;31(8):1311-8.
6. Brady CA, Attardi LD. p53 at a glance. J Cell Sci. 2010 Aug 1;123(Pt 15): 2527-32.

Appendix

96-well Culture Plate Modifications

Successful adaptation to a 96-well culture format is dependent on cell type and target. The number of cells to be plated per well should be determined for each cell type. General recommended plating concentrations range from 1 x 10^4–10^5 cells per well. These numbers are provided as a guide; the optimal concentrations will vary depending on cell line used.

Suspension Cells

Many cell types can be lysed without removing growth medium. For flat bottom plates, design the experiment so that the final suspension cell volume per well is such that a concentrated complete lysis buffer (prepared by the user) can be added to the well to achieve a final a 1X lysis buffer concentration in the well. For example, 40 µL of 5X complete lysis buffer added to a well containing 160 µL of cell culture medium would provide a 1X concentration of complete lysis buffer.

For conical microwell plates, perform lysis by pelleting the cells, removing most of the growth medium, and adding a constant amount of 1X complete lysis buffer.

Adherent Cells

Plate cells on tissue culture plates to reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from the microwell plate to avoid disrupting the cell monolayer. A PBS wash step is not required and can introduce variability as cells may detach during the wash step. Add 100 µL 1X complete lysis buffer per well. Lysis volume may be modified for different cell types or applications.

Cell lysis time should be determined by the end user. Some targets are immediately available for detection. Other targets may require an incubation step at room temperature or on ice with gentle agitation.

Carefully pipet cell lysate onto prepared capture plate and proceed with assay protocol.

It is important to transfer a constant volume and to avoid pipetting too vigorously, as the introduction of air bubbles may result.

Summary Protocol

MSD 96-well MULTI-ARRAY Phospho-p53 (Ser15) Assay Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the MULTI-ARRAY Phospho-p53 (Ser15) Assay.

Step 1: Block Plate and Prepare Samples

Add 150 µL/well of blocking solution-A.
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.
Prepare complete lysis buffer just prior to sample dilution.
Prepare positive and negative cell lysates and keep on ice until use.

Step 2: Wash and Add Sample

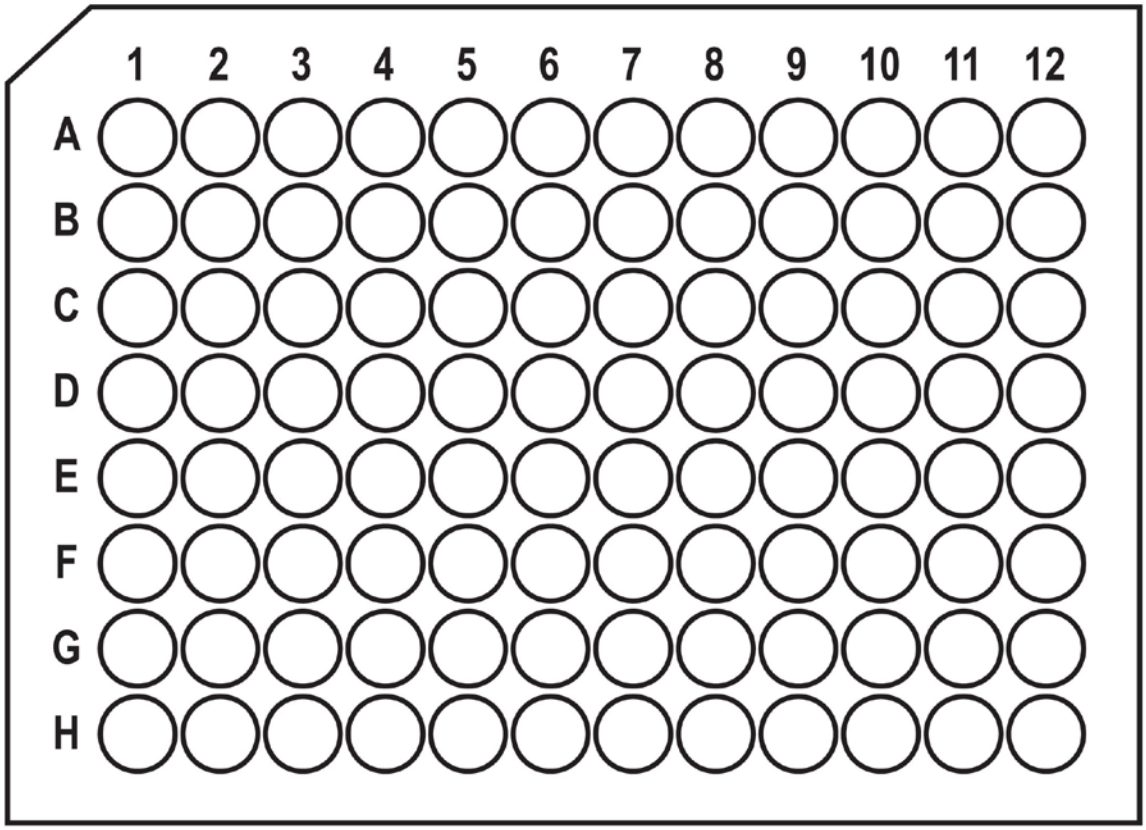
Wash plate 3 times with 300 µL/well of Tris Wash Buffer.
Dispense 25 µL/well of sample.
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of Tris Wash Buffer.
Dispense 25 µL/well 1X detection antibody solution.
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with 300 µL/well of Tris Wash Buffer.
Dispense 150 µL/well 1X Read Buffer T.
Analyze plate on SECTOR Imager within 5 minutes of read buffer addition.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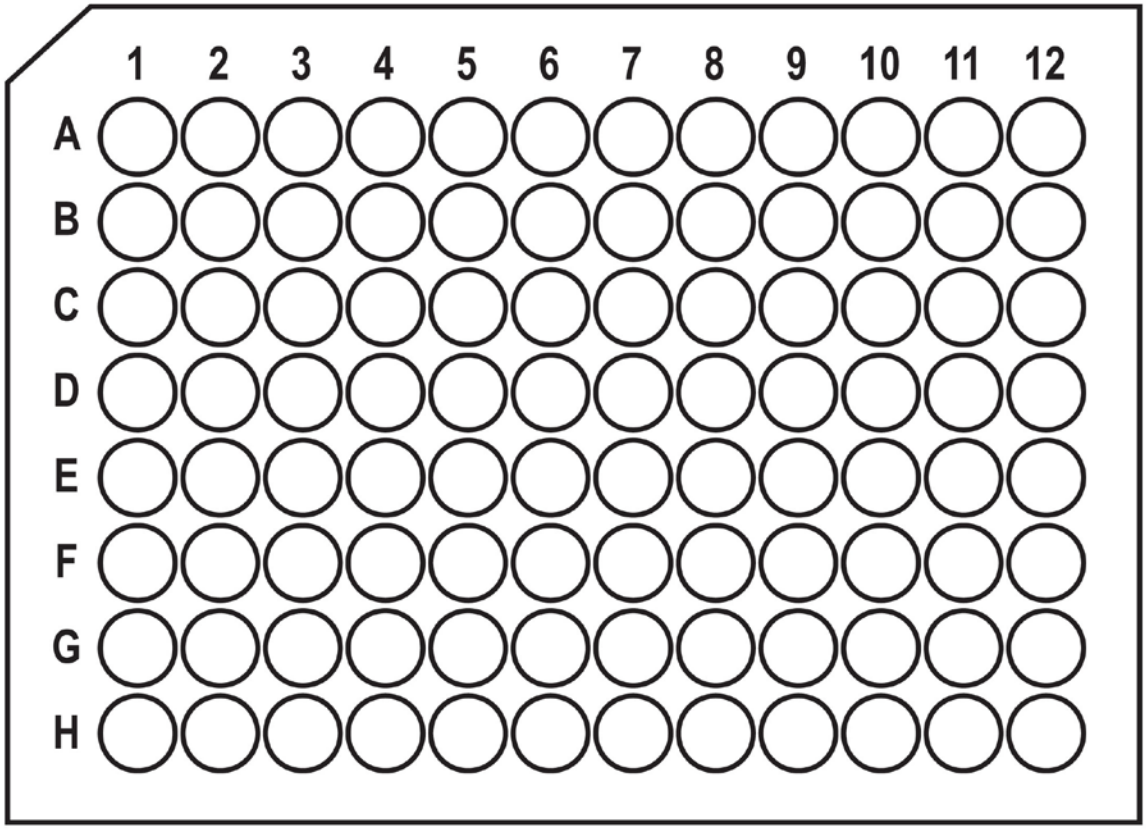
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