MSD® MULTI-SPOT Assay System

Phospho(Ser240/244)/Total S6RP Assay

Whole Cell Lysate Kit

Catalog Number

5-Plate Kit K15139D-2

www.mesoscale.com®

MSD Phosphoprotein Assays

Phospho(Ser240/244)/Total S6RP Assay

Whole Cell Lysate Kit

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MESO SCALE DISCOVERY'S unique spot patterns are a hallmark of our MULTI-ARRAY® technology, which enables the measurement of biomarkers using electrochemiluminescence (ECL) detection. In an MSD assay, specific capture antibodies for the analytes are coated in arrays in each well of a 96-well, carbon-electrode plate surface. The detection system uses patented SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT® plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable, and are nonradioactive. Additionally, only labels near the electrode surface are detected, enabling nonwashed assays.

One of the advantages of MSD assays is the minimal sample volume required compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, up to ten different biomarkers can be analyzed simultaneously using as little as 10–25 µL of the sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Additionally, the simple and rapid protocols of MSD assays provide powerful tools to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell-signaling pathways, and other applications as well as a variety of plates and reagents for assay development.

Phospho(Ser240/244)/Total S6RP

S6 Ribosomal Protein (S6RP) is the S6 subunit of the 40S ribosome, and it functions to increase translation of mRNA containing a 5′-terminal oligopyramidine tract (5′-TOP) mRNAs with a 5'-TOP generally encode proteins involved in the translational machinery, such as proteins involved in ribosome formation. S6RP functions to control translation of proteins that are constituents of the ribosome; therefore, it helps to control overall levels of protein translation. S6RP's function is phosphorylation dependent, and S6RP is phosphorylated by P70S6K in a mitogen-dependent fashion. Residues Ser235, Ser236, Ser240, and Ser244, located within the C-terminus of S6RP, are phosphorylated and may be important for activation of S6RP. While mitogens can increase the translation of 5'-TOP mRNAs, rapamycin, and analogs of rapamycin can decrease translation of these mRNAs. Taken together, this leads to the thought that S6RP may be involved in general control of cell growth.

Principle of the Assay

The MSD phosphoprotein assays provides rapid and convenient methods 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(Ser240/244)/Total S6RP Assay uses a sandwich immunoassay format (Figure 1). MSD provides a plate that has been precoated with capture antibodies for phosphorylated S6RP (Ser240/244) and total S6RP on spatially distinct spots. The user adds the sample and a solution containing the detection antibody — anti-total S6RP conjugated with an electrochemiluminescent compound, MSD SULFO-TAG label — in 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 ECL and loads the plate into an MSD® instrument where a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of phosphorylated S6RP (Ser240/244) and total S6RP present in the sample.

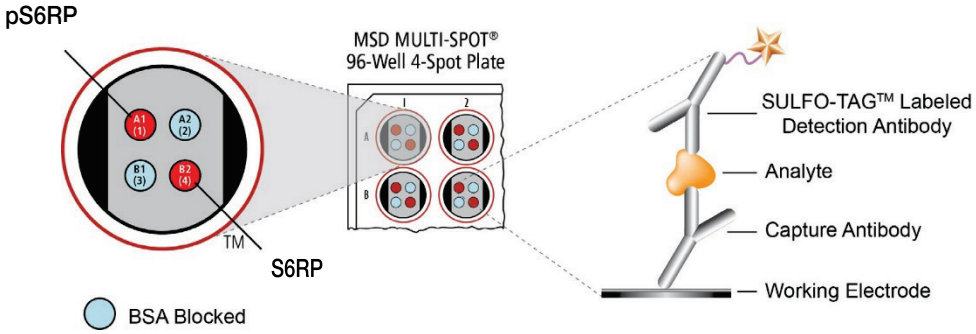

Figure 1. Spot diagram showing the placement of the analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD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

Reagents Supplied

Table 1. *Reagents Supplied*

Product Description	Catalog Number	Storage	Quantity per Kit (K15139D-2)
MULTI-SPOT 96-Well, 4-Spot Phospho(Ser240/244)/Total S6RP Plate	N45139A-1	2–8 °C	5 plates
SULFO-TAG Anti-Total S6RP Antibody (50X)	—	2–8 °C	1 vial (375 µL)
Tris Lysis Buffer (1X)	R60TX-3	2–8 °C	1 bottle (50 mL)
Tris Wash Buffer (10X)	R61TX-2	2–8 °C	1 bottle (200 mL)
Phosphatase Inhibitor I (100X)	—	2–8 °C	1 vial (0.5 mL)
Phosphatase Inhibitor II (100X)	—	2–8 °C	1 vial (0.5 mL)
Protease Inhibitor Solution (100X)	—	2–8 °C	1 vial (0.5 mL)
Blocker D-M (2%)	R93BM-2	≤–10 °C	1 vial (0.9 mL)
Blocker D-R (10%)	R93BR-2	≤–10 °C	1 vial (0.2 mL)
Blocker A (dry powder)	R93BA-4	RT	1 vial (15 g)
Read Buffer T (4X)	R92TC-3	RT	1 bottle (50 mL)

Dash (—) = not available
RT = room temperature

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 the desired throughput, capable of dispensing 10–150 µL into a 96-well, microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate rotary shaker and vortexer

Optional Material

- ❑ Akt Signaling Whole Cell Lysate Set (available for separate purchase from MSD, catalog number C1196-1)

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 local, state, and federal guidelines.

Reagent Preparation

Prepare Tris Wash Buffer

Dilute 10X stock of Tris Wash Buffer provided with the MSD kit to 1X. 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

For one plate, combine:

- ❑ 600 mg Blocker A (dry powder)
- ❑ 20 mL 1X Tris Wash Buffer
- ❑ Set aside on ice.

Prepare Antibody Dilution Buffer

For one plate, combine:

- ❑ 150 µL 2% Blocker D-M
- ❑ 30 µL 10% Blocker D-R
- ❑ 1 mL Blocking Solution
- ❑ 1.82 mL 1X Tris Wash Buffer

Set aside on ice.

Prepare Complete Lysis Buffer

To 10 mL of 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 Detection Antibody Solution

For one plate, combine:

- ❑ 2.94 mL Antibody Dilution Buffer
- ❑ 60 µL of SULFO-TAG Anti-Total S6RP Antibody (1X final concentration)

Set aside on ice.

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 precoated with the antibodies for the analytes shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., prewetting) 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 the scale of preparation and type of cells. Larger cells (e.g., NIH/3T3, or HeLa, etc.) should be lysed at concentrations of 1×10^6 to 5×10^6 cells per mL of lysis buffer. Smaller cells (e.g., Jurkat) should be lysed at concentrations of 1×10^7 to 5×10^7 cells per mL of lysis buffer.

Analysis of proteins in their activated state (i.e., phosphorylated) usually requires stimulation before cell lysis. Verification of cell stimulation and sample preparation should be performed before using this kit.

Phosphate-buffered saline (PBS) should be ice-cold before use.

Suspension Cells

Pellet cells by centrifugation at 500 × *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 1×10^7 to 5×10^7 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 10,000 × *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.

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 × *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 × *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 the Appendix for cell lysate preparation protocol modifications that accommodate the use of 96-well, culture plates.

Assay Protocol

Important: Read the entire protocol and notes before beginning the assay.

The following protocol describes the most conservative approach to achieving optimal results with the MULTI-SPOT Phospho(Ser240/244)/Total S6RPAssay. 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.

Notes:

- Solutions containing MSD Blocker A should be stored at 2–8 °C and discarded after 14 days.
- Samples, including cell lysates, etc., may be used neat or after dilution.
- MSD plates are compatible with most sample matrices. Avoid reagents that will denature the capture antibodies. For example, high concentrations of reducing agents such as DTT should be avoided. Additionally, SDS and other ionic detergents should constitute 0.1% or less in the samples applied to the wells.
- 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.
- Complete lysis buffer should be kept ice-cold during all experimental manipulations.
- Samples and standards cannot be serially diluted in the MSD plate. Use microcentrifuge tubes or a separate 96-well, polypropylene plate to prepare dilutions.
- Thaw cell lysate samples on ice, and dilute them immediately before use. Keep on ice during all manipulations, and discard all remaining thawed or unused material.
- Dilute cell lysate in Complete Lysis Buffer to a final concentration of 0.4 µg/µL. This will deliver 10 µg/well in 25 µL. A dilution series may also be prepared if desired.

STEP 1: Block Plate and Prepare Samples

- ❑ Add 150 µL of Blocking Solution to each well. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1,000 rpm) at room temperature.
- ❑ Prepare Complete Lysis Buffer just before sample dilution.
- ❑ Prepare positive and negative cell lysates (if purchased separately).

STEP 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 (300–1,000 rpm) at room temperature.
- ❑ Prepare antibody detection solution during this time.

STEP 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 (300–1,000 rpm) at room temperature. Remove the seal, wash, and read immediately.

STEP 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 an MSD instrument.

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) × 100

MULTIPLEX ASSAY FORMAT: Anti-total and anti-phospho-assay in the same well

% phosphoprotein = (2 × phospho-signal) / (phospho-signal + total signal) × 100

Notes:

- The above calculations assume that the capture antibodies on the anti-phospho and anti-total spots have very similar binding affinities (see Table 2 and Table 3).
- 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.
- The denominator is (phospho + total) because this represents the total of all the analyte captured on both of the spots.
- If the percent phosphorylation is greater than 100%, the distribution factor in the numerator may be adjusted to less than 2X such that the percent phosphorylation with the control lysates is 100%.

Table 2. Phospho-Protein Assay

Phospho-Protein Assay							
Lysates (µg)	Positive Control Lysate			Negative Control Lysate			Positive/ Negative Ratio
	Average Signal	Standard Deviation	%CV	Average Signal	Standard Deviation	%CV	
0	245	4	1.4	242	6	26.0	—
5.0	19,235	2,342	12.2	461	3	0.6	42

Dash (—) = not applicable

Table 3: Total Protein Assay

Total Protein Assay							
Lysates (µg)	Positive Control Lysate			Negative Control Lysate			Positive/Negative Ratio
	Average Signal	Standard Deviation	%CV	Average Signal	Standard Deviation	%CV	
0	561	18	3.2	569	19	3.4	—
5.0	7,304	1,227	16.8	14,530	585	4.0	0.5

Dash (—) = not applicable

We know the percent phosphoprotein = [(2 × phospho-signal) / (phospho signal + total signal)] × 100

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

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

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

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

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

Typical Data

Representative results for the MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay are illustrated in Figure 2. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-S6RP and total S6RP antibodies, and they are also shown below for comparison. Growing, low-density Jurkat cells were treated with LY294002 (50 µM, 2.5 hours) (negative) or PMA (200 nM, 15 minutes) (positive). Whole-cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-S6RP (Ser240/244) and anti-total S6RP antibodies on spatially distinct electrodes within a well. Phosphorylated and total S6RP were detected with anti-total S6RP antibody conjugated with MSD SULFO-TAG reagent.

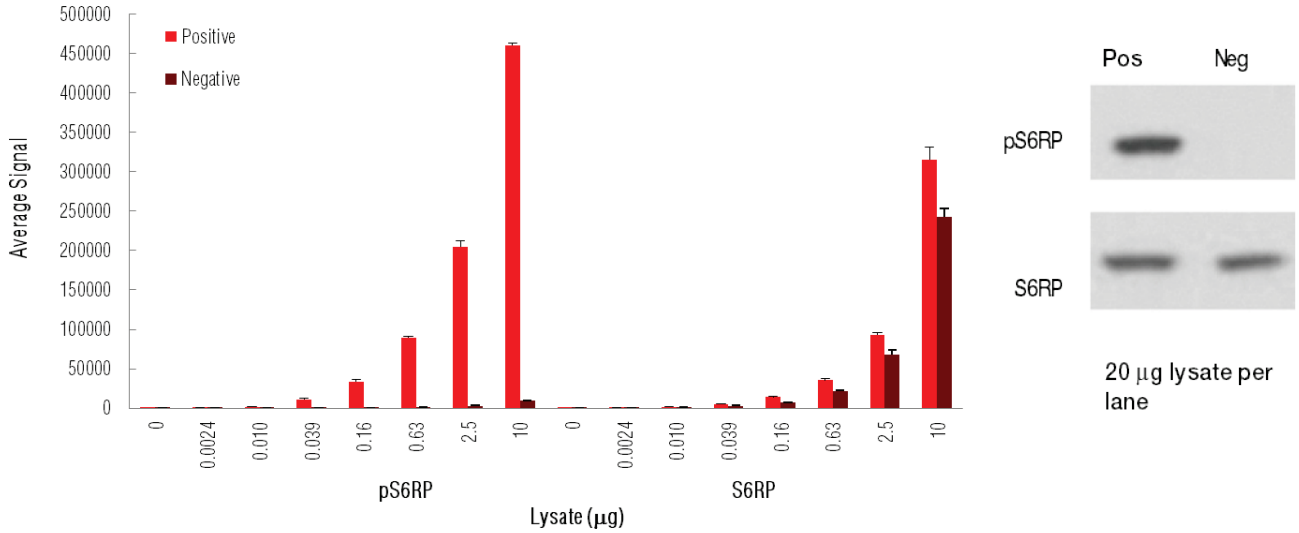

Figure 2. Sample data generated with the MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay.

Note: Increased signal for phosphorylated S6Rp was observed with only pS6RP-positive cell lysate. Total S6RP signal increased throughout the titration of both pS6RP positive and negative cell lysates. The Phospho(Ser240/244)/Total S6RP Assay provided a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pS6RP positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay are presented in Table 4.

Table 4. Positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Ser240/244)/Total S6RP

Phospho-Status	Lysate (µg)	Positive			Negative			P/N
		Average Signal	SD	%CV	Average Signal	SD	%CV	
pS6RP	0	67	19	28.8	67	19	28.8	—
	0.0024	582	48	8.3	162	19	11.4	3.6
	0.010	1910	103	5.4	245	29	11.8	7.8
	0.039	11119	1180	10.6	334	47	14.0	33
	0.16	33531	2335	7.0	406	46	11.3	83
	0.63	89400	1566	1.8	1025	34	3.3	87
	2.5	204891	7343	3.6	3106	251	8.1	66
	10	460251	2625	0.6	9479	549	5.8	49
S6RP	0	85	21	24.4	85	21	24.4	—
	0.0024	442	29	6.6	325	17	5.1	1.4
	0.010	1698	219	12.9	833	124	14.9	2.0
	0.039	5375	244	4.5	2886	305	10.6	1.9
	0.16	14441	494	3.4	7051	699	9.9	2.0
	0.63	35726	2063	5.8	21425	1689	7.9	1.7
	2.5	93210	2457	2.6	68426	4909	7.2	1.4
	10	315442	15384	4.9	242919	10163	4.2	1.3

SD = standard deviation
Dash (—) = not applicable
P/N = positive to negative ratio

Assay Components

The capture and detection antibodies used in this assay are listed in Table 5. They cross-react with human, mouse, and rat whole cell lysates.

Table 5. Capture and detection antibodies

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
Phospho-S6RP	Mouse Monoclonal	Rabbit Monoclonal
S6RP	Mouse Monoclonal	Rabbit Monoclonal

Limitations of the Procedure

The following points should be noted with the MULTI-SPOT Phospho(Ser240/244)/Total S6RPAssay 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 before use, and any remaining thawed material should be subsequently discarded.

Companion Products

Companion kits and sizes are listed in Table 6A, 6B, and 6C along with their catalog numbers.

Table 6A. *MULTI-ARRAY Phospho-S6RP (Ser240/244) Assay*

Kit Size	Catalog Numbers
1 plate	K150DGD-1
5 plates	K150DGD-2
20 plates	K150DGD-3

Table 6B*. MULTI-ARRAY Total S6RP Assay*

Kit Size	Catalog Numbers
1 plate	K150DHD-1
5 plates	K150DHD-2
20 plates	K150DHD-3

Table 6C. *MULTI-SPOT Akt Signaling Panel II (pAkt, pp70S6K, pGSK-3beta, pS6RP)*

Kit Size	Catalog Numbers
1 plate	K150DHD-1
5 plates	K150DHD-2
20 plates	K150DHD-3

Appendix

96-Well, Culture Plate Modifications

Successful adaptation to a 96-well culture format is cell type- and target-dependent. The number of cells to be plated per well should be determined for each cell type. General, recommended plating concentrations for adherent cells range from 1×10^4 to 5×10^4 cells per well and approximately 2×10^6 cells per mL (50–75 µL per well) for suspension cells. These numbers are provided as a guide, and the optimal concentrations will vary depending upon the cell line used.

Suspension Cells

For flat-bottom plates, experiments should be designed such that the final volume per well is 50–75 µL. Perform cell lysis using a 4X Complete Lysis Buffer concentrate supplemented with protease and phosphatase inhibitors at 4X concentrations. Add 4X Complete Lysis Buffer directly to cells in the growth medium for a final 1X concentration in the well.

Note: With some effort, a 10X Complete Lysis Buffer can also be prepared.

(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 biologically treated tissue culture ware such as Corning BioCoat Plates (Tewksbury, MA) reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from microwell plate. A PBS wash step is not required and can introduce variability. Add 50–100 µL 1X Complete Lysis Buffer per well.

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, 45 °C, or on ice with gentle agitation.

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

It is important to transfer a constant volume and avoid pipetting too vigorously because the introduction of air bubbles may result. Targets can be captured from a volume greater than 25 µL.

Summary Protocol

MSD 96-Well, MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the MULTI-SPOT Phospho(Ser240/244)/Total S6RP Assay.

STEP 1: Block Plate and Prepare Samples

- ❑ Add 150 µL/well of Blocking Solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1,000 rpm) for 1 hour. Prepare Complete Lysis Buffer just before sample dilution.
- ❑ Prepare positive and negative cell lysates and keep on ice until use.

STEP 2: Wash and Add Sample

- ❑ Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Dispense 25 µL/well samples.
- ❑ Incubate at room temperature with vigorous shaking (300–1,000 rpm) for 1 hour.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Dispense 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1,000 rpm) for 1 hour.

STEP 4: Wash and Read Plate

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

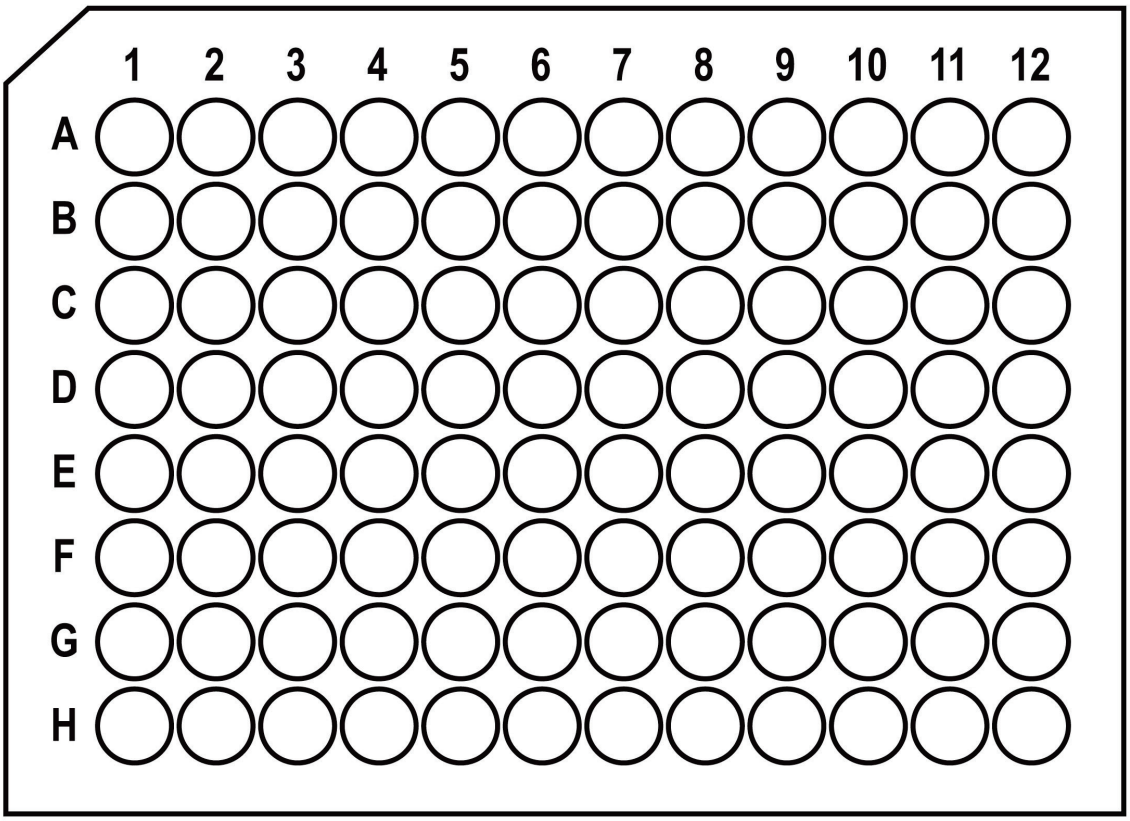

Figure 3. Plate layout. A similar plate layout can be created in Excel and easily imported into DISCOVERY WORKBENCH® software.