Meso Scale Discovery®

MULTI-SPOT® Assay System

Whole Cell Lysate Kit - Phospho (Ser 473)/Total Akt Assay

K15100D-1 K11100D-1
K15100D-2 K11100D-2
K15100D-3 K11100D-3

MSD MULTI-SPOT Biomarker Detection

Whole Cell Lysate Kit

Phospho (Ser 473)/Total Akt

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

V7 2010Feb

For Research Use Only.
Not For Use in Diagnostic Procedures.

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Ordering Information

ordering information

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Meso Scale Discovery

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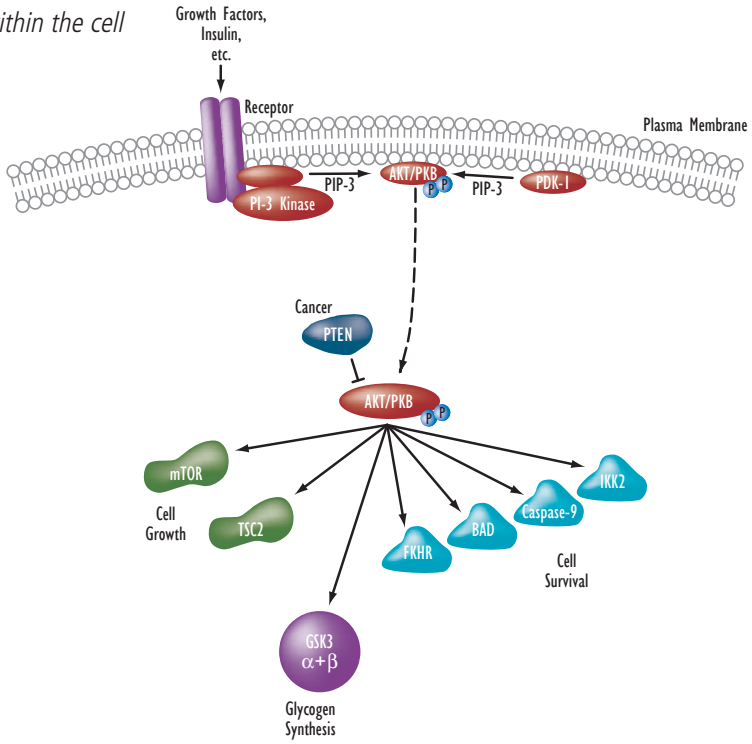
Introduction

Akt, also known as protein kinase B (PKB) or Rac, is a serine/threonine kinase that is of significant interest in pharmaceutical research due to its implicated role in cell growth, cell survival, cancer, and diabetes. The three mammalian isoforms, Akt1, Akt2, and Akt3, contain an amino-terminal pleckstrin homology (PH) domain, central catalytic domain and carboxy-terminal regulatory region. The PH domain of Akt binds the lipid products PI-3,4-P2 and PI-3,4,5-P3 generated by phosphoinositide 3-kinase (PI3K), which results in the translocation of Akt to the plasma membrane. This event causes a conformational change that facilitates the phosphorylation of Akt on Thr^{308} and Ser^{473} by 3-phosphoinositide-dependent kinase-1 (PDK1) and possibly by other additional kinases. In turn, Akt phosphorylates a wide variety of targets. Akt affects cell growth by the phosphorylation of mTOR and the phosphorylation and inactivation of the mTOR inhibitor tuberin (TSC2). Activation of mTOR causes increased translation through several additional downstream signaling events. Akt promotes growth factor-mediated cell survival by the inhibition of apoptosis through several pathways, including the inactivation of BAD, caspase-9, IKKα and the forkhead transcription factors. The tumor suppressor gene PTEN is an upstream negative regulator of Akt, and when inactivated leads to the delay of cell death due to the overexpression of Akt. This anti-apoptotic effect of Akt overexpression has been observed in breast, pancreatic, and ovarian cancer cells. Akt regulates glycogen synthesis through the inactivation of glycogen synthase kinase 3-α and β, and may play a role in glucose uptake following insulin stimulation.

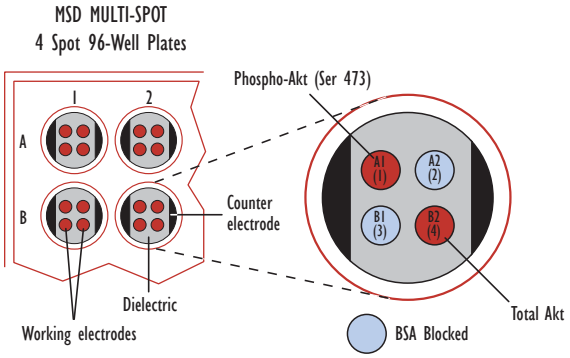
Figure 1: *Akt signaling within the cell*



Principle of the Assay

MSD® biomarker detection assays provide a rapid and convenient method for measuring the total and phosphorylated levels of protein targets within a single small-volume sample. These 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. Our duplex assay for phospho- and total Akt uses sandwich immunoassays (Figure 2). MSD provides a plate that has been pre-coated with capture antibodies (antibodies for phospho- (Ser 473) and total Akt). The user adds the sample and a solution containing the labeled detection antibody (anti-total Akt antibody labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label) over the course of one or more incubation periods. Phosphorylated and unphosphorylated Akt in the sample bind to the capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound total and phospho-Akt 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 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 the emitted light to afford a quantitative measure of the amount of total and phosphorylated Akt present in the sample.

Figure 2: *MSD MULTI-SPOT Assay*



The SECTOR Imager data file will identify spots according to their well location, not by the coated capture antibody name.

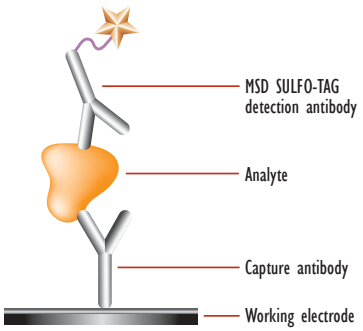


Diagram of Completed Reaction on Spots 1 and 4

III Reagents Supplied

reagents supplied

Material	Storage
Blocker A (dry powder)	RT
Read Buffer T (with surfactant), 4X	RT
MULTI-SPOT 96-well Plate (Phospho (Ser 473)/Total Akt) ► ***See Figure 2 for Spot Identification***	2-8ºC
SULFO-TAG Anti-Total Akt 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

IV Required Materials and Equipment- not supplied

Required Materials and Equipment- not supplied

- Deionized water for diluting concentrated buffers
- 250 mL bottle (1)
- 50 mL tube (2)
- 15 mL tube (1)
- Adhesive plate seals
- Microtiter plate shaker
- Microcentrifuge tubes (various) for preparing serial dilutions of samples
- Appropriate liquid handling equipment for desired throughput, capable of accurately dispensing 25 and 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer, or other efficient multi-channel pipetting equipment

V Optional Material- not supplied

Optional Materials - not supplied

- Phospho-Akt (Ser 473) Whole Cell Lysate Set (C11CA-1)

VI Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and a lab coat 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.

VII Reagent Preparation

reagent preparation

1. **Tris Wash Buffer:**

   Dilute 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 250 mL per plate is required - more if using an automatic plate washer.

   Combine (per plate):
   - 25 mL 10X Tris Wash Buffer
   - 225 mL deionized water

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

2. **Blocking Solution:**

   Combine (per plate):
   - 600 mg Blocker A (dry powder)
   - 20 mL 1X Tris Wash Buffer

3. **Antibody Dilution Buffer:**

   Combine (per plate):
   - 1 mL Blocking Solution
   - 2 mL Tris Wash Buffer

   Set aside on ice.

4. **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):
   - 200 µL Protease Inhibitor Solution (50X stock)
   - 100 µL Phosphatase Inhibitor I (100X stock)
   - 100 µL Phosphatase Inhibitor II (100X stock)

   Keep Complete Lysis Buffer on ice until use.

5. Detection Antibody Solution:

Combine (per plate):

- 2.94 mL Antibody Dilution Buffer
- 60 µL 50X SULFO-TAG Anti-Total Akt Antibody (1X final concentration)

6. 1X Read Buffer:

Combine (per plate):

- 15 mL deionized water
- 5 mL 4X Read Buffer T, with surfactant

VIII Sample Preparation and Storage

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 >1 mM) should be avoided. Please contact MSD Customer Support with any questions regarding lysate preparation options.

All manipulations should be performed on ice. Prepare Complete Lysis Buffer. 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.

Cells should be prepared as desired to activate target protein.

Phosphate Buffered Saline (PBS) should be chilled ice-cold.

Suspension cells

Pellet cells by centrifugation at 500 x g at 4ºC for 3 minutes. 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 10,000 x g, at 4ºC 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 -80ºC.

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 and 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 4ºC. 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 4ºC 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 -80ºC.

Refer to Appendix I (page 16) for cell lysate preparation protocol modifications that accommodate the use of 96-well culture plates.

IX Protocol

protocol

Read entire protocol prior to beginning assay.
Please contact MSD Customer Support with any questions.

The following protocol describes the most conservative approach to achieving optimal results with the MULTI-SPOT Phospho (Ser 473)/Total Akt 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 incubation and wash steps. Samples may be prepared for testing in the manner outlined in Sample Preparation and Storage, section VIII.

STEP 1: Block Plate and Prepare Samples.

a) Add 150 µL/well of Blocking Solution.

b) Incubate with shaking for 1 hour at room temperature. Prepare Complete Lysis Buffer and dilute samples during this time.

Note: 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 (e. g. high concentrations of reducing agents such as DTT, and also SDS and other ionic detergents should be 0.1% or less in the sample applied to the well).

Note:

Solutions containing MSD Blocker A should be stored at 4ºC and discarded after 14 days.

Plates may also be blocked overnight at 4ºC.

- 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: (if provided with kit)

1) Thaw cell lysate samples on ice and dilute immediately before use. Keep on ice during all manipulations and discard all remaining thawed unused material.
2) Dilute cell lysate in Complete Lysis Buffer to a final concentration of 0.8 µg/µL. This will deliver 20 µg/well in 25 µL. A dilution series may also be prepared if desired.
3) Keep diluted cell lysate on ice until use.

d) Wash plates four times with at least 150 µL/well Tris Wash Buffer.

Note:

Complete Lysis Buffer should be prepared the day of use and then discarded. It 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.

STEP 2: Add Samples and Prepare Detection Antibody Solution.

a) Dispense 25 µL/well of samples.

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

c) Wash plates four times with at least 150 µL/well Tris Wash Buffer.

Note:

Shaking a 96-well MSD MULTI-ARRAY® or MULTI-SPOT plate during an incubation step will 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.

STEP 3: Add Detection Antibody.

a) Add 25 µL/well of Detection Antibody Solution.

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

c) Wash plates four times with at least 150 µL/well Tris Wash Buffer.

Note:

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

STEP 4: Read Plate.

a) Carefully add 150 µL/well of diluted Read Buffer T, with surfactant, avoiding the introduction of any bubbles.

b) Analyze with SECTOR Imager:

1. Double click on DISCOVERY WORKBENCH® icon on computer desktop (if not already open).
2. Click the SECTOR Imager icon in upper left corner of screen (if not already open to plate reading screen).
3. From the pull down menu select "Read From Barcode."
4. If only reading one plate check "Return Plate to Input Stack."
5. Check the box and enter number of plates to be read.
6. Click the "Run" button.
7. Check the box to export default data file.
8. If desired, make selections to export a custom data file.
9. Browse and select the location to export data files.
10. Click OK to initiate the run.
11. Data will be automatically saved in the software database and text versions of the requested data files exported to the folder designated.

Note:

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

Plates 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.

Note: Once the assay is fully developed and producing desired results, the protocol listed above can be streamlined by combining the lysate and antibody incubation steps and removing the corresponding washes. A checklist summary protocol is seen below with a sample experimental plate layout.

- ☐ Add blocking solution and incubate.
- ☐ Wash.
- ☐ Add samples and incubate.
- ☐ Wash.
- ☐ Add detection antibody solution and incubate.
- ☐ Wash.
- ☐ Add Read Buffer and analyze plate.

Cell Lysate (µg)		Control Samples								Test Samples			
		pAkt Positive				pAkt Negative				Unknown			
		1	2	3	4	5	6	7	8	9	10	11	12
0	A												
	B												
5	C												
	D												
10	E												
	F												
20	G												
	H												

Figure 3: *Sample assay plate layout. MSD Control Cell Lysates that should produce signal for phosphorylated and total Akt are shaded in yellow and total Akt only are shaded in green.*

X Calculation of Results

calculation of results

The percent phosphoprotein in a sample can be calculated using independent MSD total and phospho singleplex phosphoprotein assays or MSD total/phospho multiplex phosphoprotein assays. MSD has optimized the amount of capture antibody used in multiplex assays to account for differences in binding affinities between anti-phospho and anti-total capture antibodies, as multiple capture antibodies may compete for the same analyte in a multiplex assay format.

INDEPENDENT ASSAY FORMAT: Anti-Total Singleplex and Anti-Phospho Singleplex using the same detection antibody

% 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

Note: The numerator is 2X the phospho signal because the phosphorylated species is captured by both the phospho-specific and the phosphorylation state-independent capture antibodies, and the denominator is "phospho + total" signal because the true total is all of the material captured on both spots.

The following points should be noted when calculating percent phosphoprotein using MSD assays:

- The capture antibodies used in MSD multiplex assays may differ in their weak binding to abundant proteins in the test lysate, therefore each assay in the well may not be linear over the same concentration range.
- Different protein targets may vary greatly in abundance within a particular sample, therefore the establishment of a linear range for each target is recommended.

Sample Data and Calculations for Each Assay Format:

Jurkat cell lysates were untreated or treated with the inhibitor LY294002. Capture antibodies used for plate coating are indicated.

For demonstration purposes, calculations for percent phosphoprotein are shown here using data from MSD MULTI-SPOT Phospho-Akt, Total Akt, and Phospho/Total Akt Assays.

INDEPENDENT ASSAYS

Phospho-Akt Capture Antibody								
Jurkat lysates	pAkt Positive			pAkt Negative			P-N	P/N
(µg)	Ave	StdDev	%CV	Ave	StdDev	%CV		
0	121	6	5	104	1	1		
0.3	856	66	8	144	14	10	712	5.9
0.6	1370	87	6	169	13	8	1201	8.1
1.3	2448	10	0	209	88	42	2239	11.7
2.5	4402	211	5	260	106	41	4142	16.9
5	8925	439	5	455	59	13	8470	19.6
10	16618	1653	10	752	83	11	15866	22.1
20	34590	3630	10	1103	64	6	33487	31.4

Figure 4: *Phospho-Akt capture alone.*

Total Akt Capture Antibody								
Jurkat lysates	pAkt Positive			pAkt Negative			P-N	P/N
(µg)	Ave	StdDev	%CV	Ave	StdDev	%CV		
0	243	47	19	218	8	4		
0.3	1724	132	8	2588	165	6	-865	0.7
0.6	3552	69	2	5489	453	8	-1938	0.6
1.3	6530	115	2	9626	195	2	-3097	0.7
2.5	11577	242	2	18481	75	0	-6904	0.6
5	19820	1080	5	31255	77	0	-11435	0.6
10	34149	474	1	50096	58	0	-15948	0.7
20	45475	505	1	63923	1044	2	-18448	0.7

Figure 5: *Total Akt capture alone.*

MULTIPLEX ASSAY

Phospho-Akt Capture Antibody								
Jurkat lysates	pAkt Positive			pAkt Negative			P-N	P/N
(µg)	Ave	StdDev	%CV	Ave	StdDev	%CV		
0	89	16	18	112	16	14		
0.3	584	87	15	158	15	9	426	3.7
0.6	983	170	17	169	21	12	815	5.8
1.3	1373	208	15	269	12	4	1105	5.1
2.5	3124	742	24	294	28	9	2830	10.6
5	6087	3088	51	485	11	2	5602	12.6
10	15169	3718	25	732	60	8	14438	20.7
20	29258	4939	17	1173	189	16	28085	25.0

Total Akt Capture Antibody								
Jurkat lysates	pAkt Positive			pAkt Negative			P-N	P/N
(µg)	Ave	StdDev	%CV	Ave	StdDev	%CV		
0	220	44	20	243	44	18		
0.3	1705	255	15	3241	255	8	-1537	0.5
0.6	3286	89	3	5853	89	2	-2567	0.6
1.3	5629	1109	20	10390	1109	11	-4762	0.5
2.5	11701	1050	9	19325	1050	5	-7624	0.6
5	21189	3072	14	34547	3072	9	-13359	0.6
10	33731	4094	12	52575	4094	8	-18844	0.6
20	44540	14	0	71846	14	0	-27307	0.6

Figure 6: *Phospho Akt and total Akt in the same well.*

CALCULATION OF PERCENT PHOSPHOPROTEIN BY EACH METHOD

Jurkat lysates	% pAkt in pAkt Positive Lysates			% pAkt in pAkt Negative Lysates		
(µg)	alone		multi	alone		multi
0						
0.3	50		51	6		9
0.6	**39**		**46**	**3**		**6**
1.3	**37**		**39**	**2**		**5**
2.5	**38**		**42**	**1**		**3**
5	**45**		**45**	**1**		**3**
10	49		62	2		3
20	76		79	2		3

Figure 7: *Independent and multiplex calculation of percent phosphoprotein in a cell lysate titration. Untreated Jurkat cell lysate is expected to express phosphorylated Akt. Treatment with LY294002 inhibitor is expected to inhibit phosphorylated Akt production but not affect total Akt levels.*

XI Limitations of Procedure

limitations of procedure

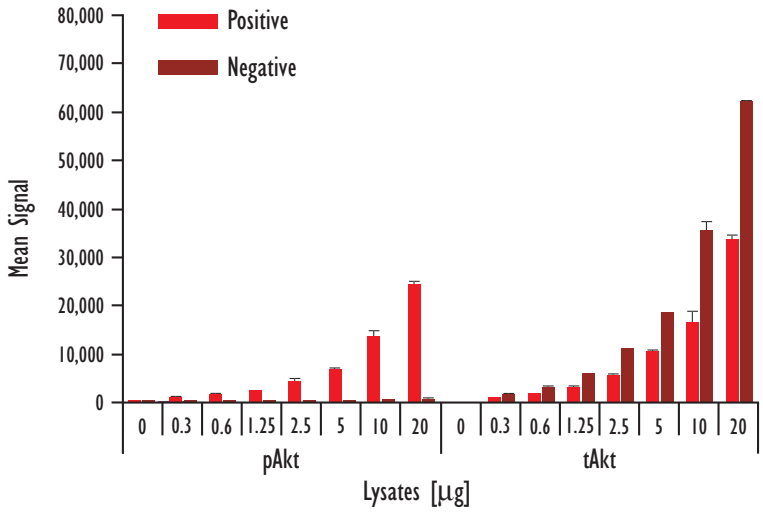
The following points should be noted with the MULTI-SPOT Phospho (Ser 473)/ Total Akt Assay in order to maximize assay sensitivity and performance:

- 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 subsequently discarded.

XII Typical Data

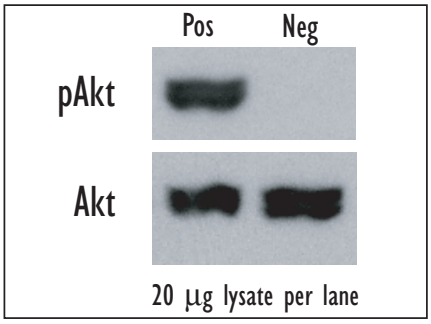
typical data

Typical results for the MULTI-SPOT Phospho (Ser 473)/Total Akt Assay are illustrated in Figures 8-10 below. Please note that these signal and ratio values are provided for demonstration only and individual results may vary depending upon samples tested.



Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM; 2.25 hr) (negative). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-Akt antibody and anti-total-Akt antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total Akt were detected with anti-total-Akt antibody labeled with MSD SULFO-TAG reagent.

Figure 8: *Sample data generated with the MSD MULTI-SPOT Phospho (Ser 473)/Total Akt Assay. Increased signal for phosphorylated Akt was observed with only pAkt positive cell lysates as total Akt signal increased throughout the titration of both pAkt positive and negative cell lysates. The MSD MULTI-SPOT Phospho (Ser 473)/Total Akt Assay provides a quantitative measure of the data obtained with the traditional Western blot.*



Lysates were prepared as described in Section VIII, Sample Preparation and Storage, from Jurkat cells treated as outlined in Figure 8. Western Blot analysis of each lysate type was performed with phospho (Ser 473)-specific and total Akt antibodies.

Figure 9: *Western Blot analysis of cell lysate. The signal generated in the MULTI-SPOT Assay for 20 µg of cell lysates (Figure 8) is consistent with the qualitative Western blot results.*

	Lysates (µg)	pAkt Positive			pAkt Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pAkt	0	368	122	33	286	54	19	
	0.3	1,659	118	7	304	46	15	5.5
	0.6	2,598	297	11	396	23	6	6.6
	1.25	3,908	139	4	392	14	3	10.0
	2.5	6,875	935	14	543	18	3	12.7
	5	10,724	933	9	676	39	6	15.9
	10	22,151	1,760	8	849	113	13	26.1
	20	39,455	761	2	1,239	103	8	31.8
tAkt	0	109	10	9	101	8	8	
	0.3	1,462	57	4	2,737	107	4	0.5
	0.6	2,843	131	5	5,092	230	5	0.6
	1.25	5,070	300	6	9,407	239	3	0.5
	2.5	9,114	337	4	17,709	86	0	0.5
	5	16,738	811	5	29,691	51	0	0.6
	10	26,538	3,884	15	57,072	3,114	5	0.5
	20	54,182	1,457	3	99,970	593	1	0.5

Figure 10: *Lysate titration data for pAkt positive and negative Jurkat cell lysates using the MSD MULTI-SPOT Phospho (Ser 473)/Total Akt Assay including precision and signal to background values.*

XIII References

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MULTI-ARRAY Phospho-Akt (Ser 473) Assay

MULTI-ARRAY Total Akt Assay

Phospho- and total Akt antibodies can be used in custom phosphorylated and total protein multiplex assay panels designed to meet specific research needs. Please contact MSD Customer Service for more information.

MSD Customer Service

Phone: 240-631-2522, ext. 4685
Fax: 301-990-2776
Email: sales@mesoscale.com

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
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For Research Use Only.
Not For Use in Diagnostic Procedures.

Appendix I

appendix i

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 x 10^4-5 x 10^4 cells per well, and approximately 2 x 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 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 BD BioCoat™ Cellware (Becton, Dickinson, and Company, Franklin Lakes, NJ)) to 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, while others may require an incubation step at room temperature, 4ºC, 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 avoid pipeting too vigorously, as the introduction of air bubbles may result. (Targets can be captured from a volume greater than 25 µL.)