

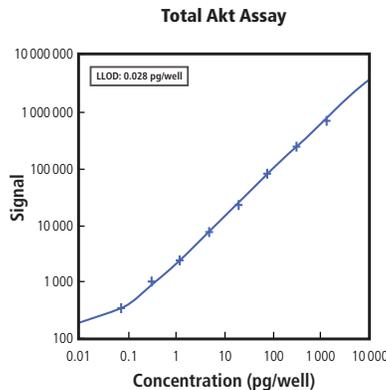
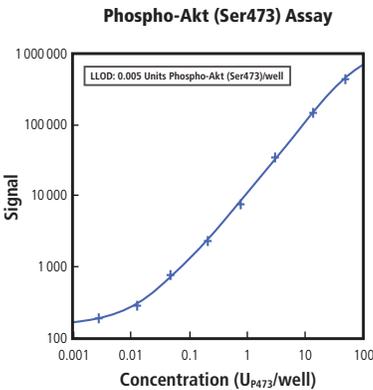
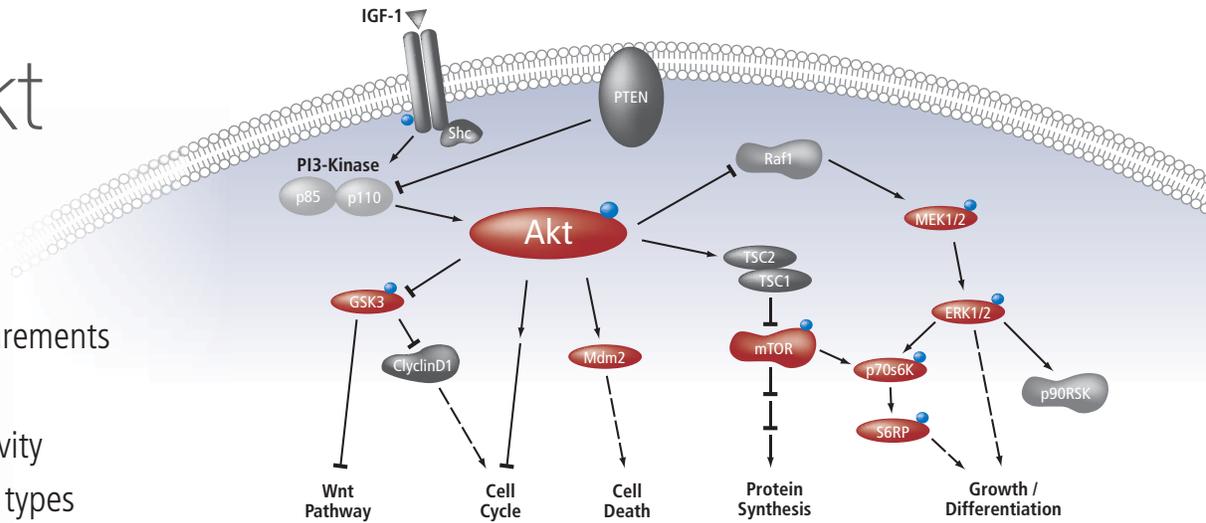


Akt Assays

For use with human, mouse, rat, and non-human primate cell and tissue lysates

Advantages of MSD[®] Akt Assays

- Quantitative Akt measurements
- Recombinant calibrator
- Industry-leading sensitivity
- Multiple cell and tissue types
- Multiple species
 - Human, mouse, rat, non-human primates
- Minimal sample volume requirements
- Simple protocols
- Customizable multiplexes

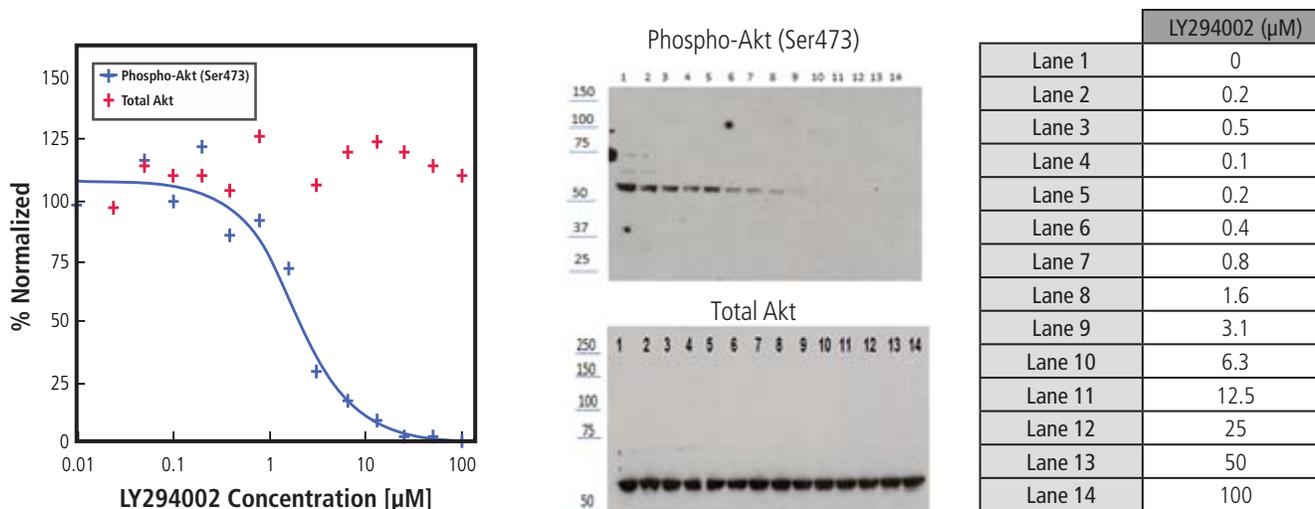


Add blocking solution to MSD plate.
 ▼ Incubate 1 hr
 Wash. Add sample or calibrator.
 ▼ Incubate 1 hr
 Wash. Add detection antibody solution.
 ▼ Incubate 1 hr
 Wash. Read plate and analyze.

For Research Use Only. Not for use in diagnostic procedures.

Pharmacological Relevance: Dose Response Analysis

Human Jurkat cells were treated with serial dilutions of the PI3K inhibitor LY294002 for 30 minutes. In the graph below, average signals from treated cells were normalized to the average signals from untreated Jurkat cells. LY294002 suppressed the phosphorylation of Akt at serine 473; levels of total Akt were not affected by the treatment. Results from Western blots are also shown.



Assay Precision

The precision of the Phospho-Akt (Ser473) Kit was measured using Akt Control Pack 1 (MSD catalog # C4115-1), which includes frozen lysates from untreated, exponentially growing Jurkat cells in two concentrations: Control 1 (total protein: 400 µg/mL; 10 µg/well) and Control 2 (total protein: 25 µg/mL; 0.63 µg/well). Control 3 was prepared by diluting Control 1 in assay diluent 100-fold to a final concentration of 4 µg/mL (0.10 µg/well). Analyte levels were measured using a minimum of 3 replicates on 18 runs over 3 days. Average intra-run %CV is the average %CV of the control replicates within an individual run. Inter-run %CV is the variability of controls across 18 runs.

	Control	Runs	Average Signal	Average $U_{P473}/\mu\text{g Lysate}$	Average Intra-run %CV	Inter-run %CV
Phospho-Akt (Ser473)	Control 1	18	44 931	0.35	4.0	7.0
	Control 2	18	1797	0.26	4.1	9.0
	Control 3	18	345	0.25	4.2	16.0
	Control	Runs	Average Signal	Average pg Akt/ $\mu\text{g Lysate}$	Average Intra-run %CV	Inter-run %CV
Total Akt	Control 1	18	238 356	35	3.2	2.8
	Control 2	18	13 000	29	3.6	12.1
	Control 3	18	2375	30	3.3	8.6

Tissue Lysate Samples

Tumor xenograft lysate samples were diluted to 400 µg/mL of total protein (10 µg/well) and tested using the Phospho-Akt (Ser473) Kit and the Total Akt Kit.

Sample Type	Measured Phospho-Akt (Ser473) ($U_{P473}/\mu\text{g Lysate}$)	Measured Total Akt (pg/ $\mu\text{g Lysate}$)
Epithelial Melanoma (human) (N=3)	0.018	10
Kidney Carcinoma (human) (N=3)	0.046	2.7
Renal Cell Carcinoma (human) (N=3)	0.13	18

Cell Lysate Samples

Test samples of immortalized mammalian cell lysates (10 µg/well) were characterized as being either positive or negative for Phospho-Akt (Ser473). These lysate sets were assayed in triplicate with the Phospho-Akt (Ser473) Kit and the Total Akt Kit. For each lysate set, the treatment and the levels of phosphorylated and total Akt/µg lysate are displayed below. The analytes were quantifiable in human, mouse, rat, and non-human primate cell lysates.

Sample Type	Positive Lysate			Negative Lysate		
	Treatment	U _{P473} /µg Lysate	pg Akt/µg Lysate	Treatment	U _{P473} /µg Lysate	pg Akt/µg Lysate
Jurkat (human)	Growing cells	0.42	31	50 µM LY294002 (2.5 hours)	0.0050	21
MCF-7 (human)	100 nM IGF-1 (20 minutes)	0.39	133	50 µM LY294002 (2.5 hours)	0.078	117
NIH3T3 (mouse)	1 mM sodium vanadate pretreatment (4 hours) followed by 33.5 nM calyculin A (30 minutes)	4.3	88	Growing cells	0.092	117
Rat L6 (rat)	1 mM sodium vanadate pretreatment (4 hours) followed by 33.5 nM calyculin A (30 minutes)	4.7	87	Growing cells	0.057	102
COS7 (non-human primate)	1 mM sodium vanadate pretreatment (4 hours) followed by 33.5 nM calyculin A (30 minutes)	1.9	62	Growing cells	0.046	52

Related Kits

MSD offers a comprehensive menu of immunoassays for analytes related to the Akt signaling pathway. The following are some of the related analytes and multiplex panels.

Related Analytes

- Phospho- and total 4E-BP1
- Phospho- and total BAD
- Cleaved and total caspase-3
- Phospho- and total EGFR
- Phospho- and total eIF4E
- Phospho- and total ErbB2
- Phospho- and total ERK1/2
- Phospho-FOXO3a
- GAPDH
- Phospho- and total GSK-3β
- Phospho- and total IGF-1R
- Phospho- and total IR
- Phospho- and total IRS-1
- Phospho- and total JNK
- Phospho-mTOR
- Ubiquitinated and total MDM2
- Phospho- and total NFκB
- Phospho- and total p38
- Phospho- and total p53
- Total p62/SQSTM1
- Phospho- and total p70S6K
- Phospho- and total PRAS40
- Phospho- and total S6RP
- Phospho- and total VEGFR2

Multiplex Panels

- Akt Signaling: p-Akt (Ser473), p-p70S6K (Thr421/Ser424), p-GSK-3β (Ser9)
- Akt Signaling (Total Protein): Akt, GSK-3β, p70S6K
- Akt Signaling Panel II: p-Akt (Ser473), p-p70S6K (Thr389), p-GSK-3β (Ser9), and p-S6RP (Ser240/244)
- Akt Signaling Panel II (Total Protein) Whole Cell Lysate Kit: Akt, p70S6K, GSK-3β, and S6RP
- EGFR Family: p-EGFR, p-ErbB2, p-IGF-1R
- Insulin Signaling: p-IGF-1R, p-IR, p-IRS-1
- Insulin Signaling (Total Protein): IGF-1R, IR, IRS-1
- MAP Kinase: p-ERK-1/2 (Thr/Tyr: 202/204; 185/187), p-JNK (Thr183/Tyr185), p-p38 (Thr180/Tyr182)
- MAP Kinase (Total Protein): ERK1/2, JNK, p38
- Phospho(Ser9)/Total GSK-3β Whole Cell Lysate Kit
- Phospho(Thr421/Ser424)/Total p70S6K Whole Cell Lysate Kit

We are continually expanding our portfolio by developing new and improved assays for disease-focused biomarkers. For the most up-to-date information and a complete listing of our products, please visit www.mesoscale.com.





Catalog Numbers

Kit Name	Species	Catalog Number		
		1 plate	5 plate	25 plate
Phospho-Akt (Ser473) Kit	Human, mouse, rat, non human primate	K150MND-1	K150MND-2	K150MND-4
Total Akt Kit	Human, mouse, rat, non human primate	K150MOD-1	K150MOD-2	K150MOD-4

Customization

The Phospho-Akt (Ser473) and the Total Akt assays have been tested with different signaling proteins in the PI3 kinase pathway. An example of assays that performed well when tested together as a multiplex is shown below. Akt assays can be ordered in combination with other analytes that are in the pathway. Contact our customer service team to design your unique custom multiplex.



Supplementary Materials

Item	Description	Catalog Number
Akt Control Pack I	Frozen Jurkat cell lysates in 2 concentrations	C4115-1
Tris Lysis Buffer	Cell lysis buffer for lysate preparation	R60TX-3 (50 mL) R60TX-2 (200 mL)
Inhibitor Pack	Phosphatase and protease inhibitor solutions for use with Tris Lysis Buffer for preparation of lysates or with Diluent 39 for preparation of additional assay diluent	R70AA-1
Akt Calibrator (20X)	Recombinant human Akt1, in vitro phosphorylated	C00MN-2
Diluent 39	Tris Lysis Buffer with carrier protein for sample and calibrator dilutions	R5ABB-3
Total Akt Antibody	SULFO-TAG conjugated Total Akt detection antibody	D20MO-2 (1 Plate) D20MO-3 (5 Plate)
Phospho-Akt (Ser473) Antibody	SULFO-TAG conjugated Phospho-Akt (Ser473) detection antibody	D20MN-2 (1 Plate) D20MN-3 (5 Plate)

Custom combinations of the assays above may be ordered online at www.mesoscale.com/V-PLEX.

Phone: 1-240-314-2795 . Fax: 1-301-990-2776

Email: customerservice@mesoscale.com

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