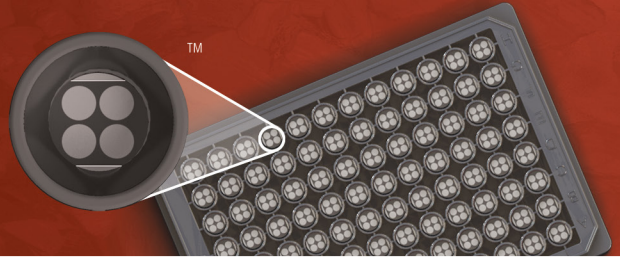
MSD® Phospho-PRAS40 (Thr246) Assay Whole Cell Lysate Kit

For quantitative determination in human, mouse, and rat whole cell lysate samples



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Phospho-PRAS40 (Thr246) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150JZD-1
5 plates	K150JZD-2
20 plates	K150JZD-3

Phospho-PRAS40 (Thr246) Whole Cell Lysate Set	
200 µg	C10JZ-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
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Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
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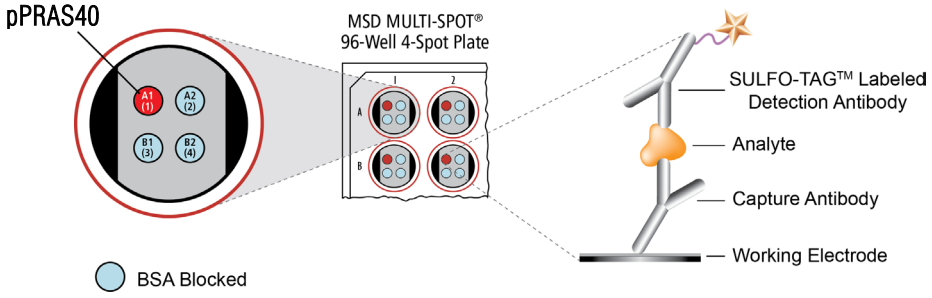


PRAS40 (Proline Rich Akt Substrate, 40kDa), also known as AKT1S1, is a recently identified, proline-rich substrate of Akt1. It contains approximately 15% proline residues as opposed to normal proteins, which have about 5%. PRAS40 has a consensus site for Akt phosphorylation located at Thr246. It has been demonstrated that there is decreased phosphorylation of PRAS40 at Thr246 in cells lacking Akt1 and Akt2. In vitro experiments with purified Akt have shown phosphorylation of PRAS40 at Thr246. PRAS40 also binds to 14-3-3 proteins when phosphorylated.[1] It has been suggested that PRAS40 might be an inhibitor of kinase activity of mTORC1.[2] Phosphorylation of PRAS40 by Akt at Thr246 relieves PRAS40 inhibition of mTORC1.[3]
PRAS40 activation is one of the early events in breast and lung cancers and its level of expression is higher in cancer cell lines (i.e., A549 and HeLa) than in normal cell lines (i.e., HEK293).[1, 4] Studies indicated that reduced PRAS40 levels increased the sensitivity of tumor cells to apoptosis. PRAS40 is also an important regulator of insulin sensitivity of the Akt-mTOR pathway and a potential target for the treatment of cancers and insulin resistance.

The MSD Phospho-PRAS40 (Thr246) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-PRAS40 (Thr246) Assay are illustrated below. 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-PRAS40 (Thr246) and total PRAS40 antibodies and are shown below for comparison.
MCF7 cells were treated with either LY294002 (50 µM, 2.5 hours) (negative) or IGF-1 (100 nM, 20 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-total PRAS40 on one of the four spatially distinct electrodes per well. Phosphorylated PRAS40 was detected with anti-phospho-PRAS40 (Thr246) antibody conjugated with MSD SULFO-TAG™ reagent.

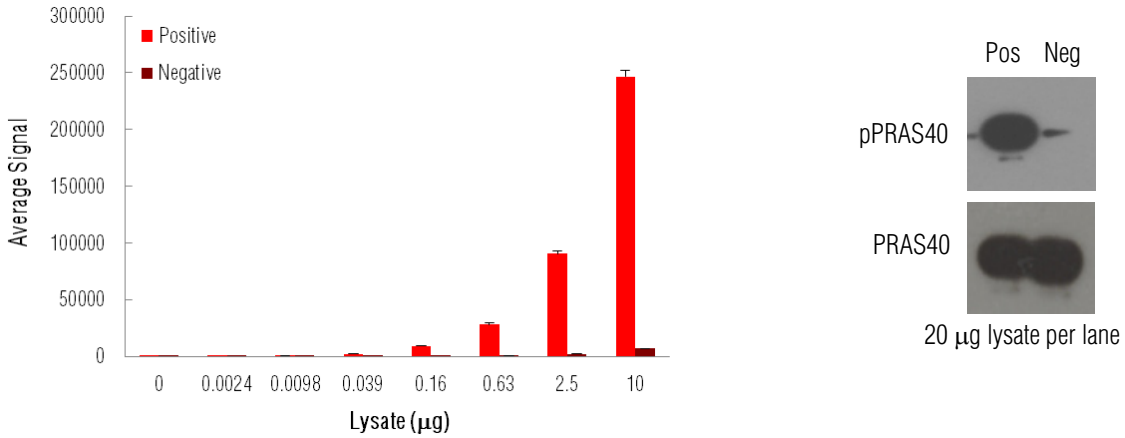


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-PRAS40 (Thr246) Assay. Increased signal is observed with the titration of pPRAS40 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-PRAS40 (Thr246) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

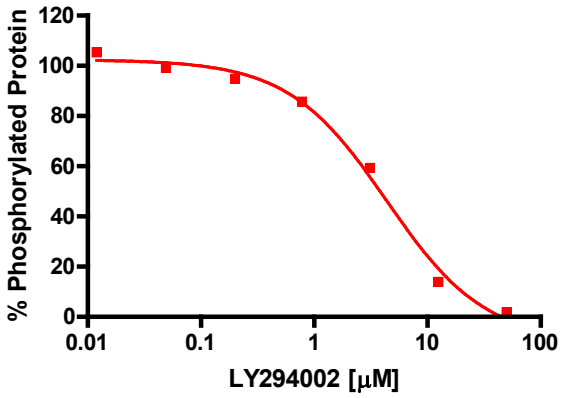
Lysate Titration

Data for pPRAS40 positive and negative MCF7 cell lysates using the MULTI-ARRAY Phospho-PRAS40 (Thr246) Assay are presented below.

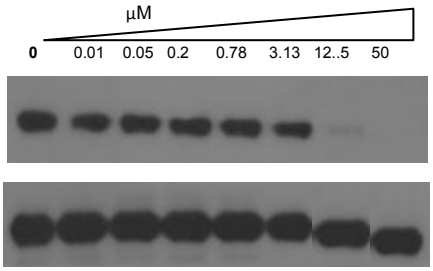
Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	86	10	11.6	83	4	4.8	
0.0024	249	9	3.6	88	6	6.8	2.8
0.0098	846	20	2.4	92	10	10.9	9.2
0.039	2569	68	2.6	126	9	7.1	20
0.16	9196	463	5	235	2	0.9	39
0.63	28456	1203	4.2	705	20	2.8	40
2.5	90876	1920	2.1	2267	116	5.1	40
10	246638	5729	2.3	7093	97	1.4	35

IC-50 Determination

The MSD Phospho-PRAS40 (Thr246) Assay was used to determine the IC-50 of LY294002, a highly selective inhibitor of phosphatidylinositol-3-kinase that blocks PI3 kinase-dependent Akt phosphorylation. MCF7 cells were plated at 4 x 10^4 cells per well in 96-well tissue culture plates in complete growth medium. Cells were grown to 70% confluence and treated with serial dilutions of LY294002 inhibitor for 2 hours. After treatment, medium was aspirated and 100 µL of complete tris lysis buffer was added per well. Plates were incubated on ice for 20 minutes and frozen at ≤-70°C. Tissue culture plates were thawed on ice and 25 µL of lysate was transferred to the MSD Phospho-PRAS40 (Thr246) plate and incubated according to the kit protocol. A separate 96-well tissue culture cell plate was subjected to identical treatments and harvested for Western blot analysis with anti-phospho-PRAS40 (Thr246) and anti-total PRAS40 antibodies.



LY294002 treatment 2 hours				
LY294002 (µM)	Average Signal	StdDev	%CV	% phosphorylated protein
0	96404	7370	7.6	100
0.012	101514	7707	7.6	105
0.049	95664	3109	3.2	99
0.20	91470	11647	12.7	95
0.78	82714	7846	9.5	86
3.1	57254	7626	13.3	59
13	13453	1118	8.3	14
50	1734	177	10.2	1.8



MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 µg/well or less without compromising speed or performance
- **Large dynamic range**: Linear range of up to five logs enables the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions
- **Minimal background:** The stimulation mechanism (electricity) is decoupled from the signal (light)
- **Simple protocols:** Only labels near the electrode surface are detected, enabling no-wash assays
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules
- **High sensitivity and precision:** Multiple excitation cycles of each label enhance light levels and improve sensitivity

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References:

1. Kovacina KS, Park GY, Bae SS, Guzzetta AW, Schaefer E, Birnbaum MJ, Roth RA. Identification of a proline-rich Akt substrate as a 14-3-3 binding partner. J Biol Chem. 2003 Mar 21;278(12):10189-94. Epub 2003 Jan 10.
2. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol. 2007 Mar;9(3):316-23. Epub 2007 Feb 4.
3. Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA, Sabatini DM. PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. Mol Cell. 2007 Mar 23;25(6):903-15.
4. Huang B, Porter G. Expression of proline-rich Akt-substrate PRAS40 in cell survival pathway and carcinogenesis. Acta Pharmacol Sin. 2005 Oct;26(10):1253-8.