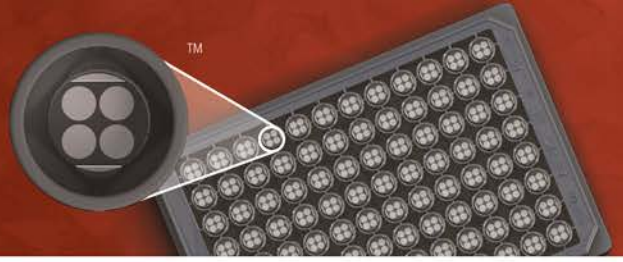
MSD® Total PRAS40 Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples



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

Catalog Numbers

Total PRAS40 Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150KRD-1
5 plates	K150KRD-2
20 plates	K150KRD-3

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

Ordering information

MSD Customer Service
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Email: CustomerService@mesoscale.com

Company Address

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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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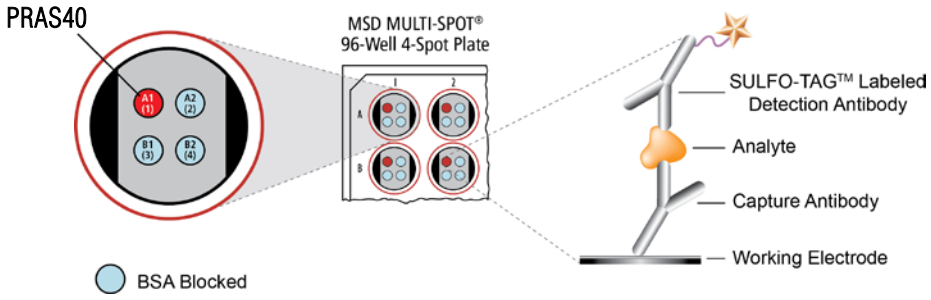

PRAS40 (Proline Rich Akt Substrate, 40kDa), also known as AKT1S1, is a proline-rich substrate of Akt1. It contains approximately 15% proline residues as opposed to normal proteins, which have about 5%. PRAS40 has a consensus site for Akt phosphorylation located at Thr246. It has been demonstrated that 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 indicate that reduced PRAS40 levels increases 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 Total PRAS40 Assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Total PRAS40 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-PRAS40 (Thr246) and total PRAS40 antibodies and are shown for comparison.
MCF-7 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. Total PRAS40 was detected with anti-total PRAS40 antibody conjugated with MSD SULFO-TAG™.

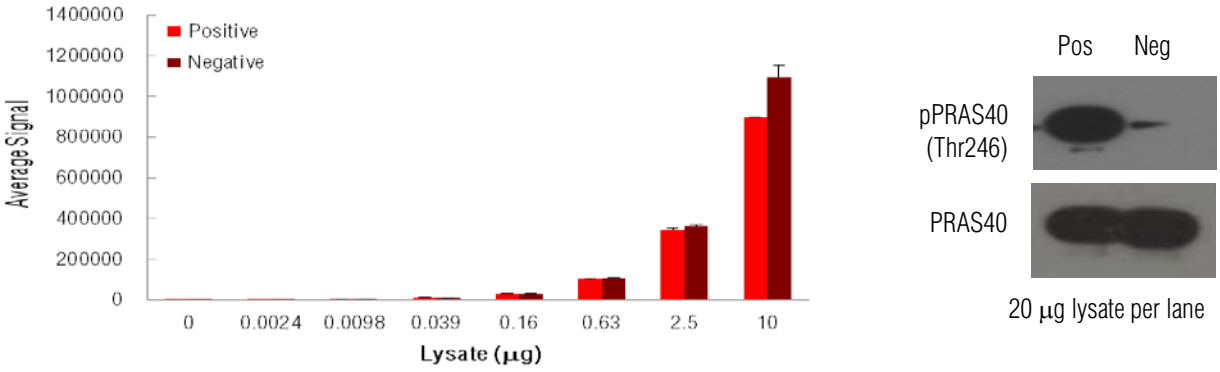

Fig. 1: Sample data generated with the MULTI-ARRAY® Total PRAS40 Assay. Increased signal is observed with the titration of both pPRAS40 positive and negative cell lysates. The Total PRAS40 Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pPRAS40 positive and negative MCF-7 cell lysates using the MULTI-ARRAY Total PRAS40 Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	622	28	4.4	609	4	0.7	
0.0024	1506	23	1.5	1505	33	2.2	1.0
0.0098	3981	13	0.3	3356	242	7.2	1.2
0.039	10545	62	0.6	9408	588	6.3	1.1
0.16	31801	1073	3.4	31130	1521	4.9	1.0
0.63	103443	2840	2.7	107936	919	0.9	1.0
2.5	343329	9135	2.7	362036	7642	2.1	0.9
10	897059	358	0.0	1093056	57219	5.2	0.8

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.
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.
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.