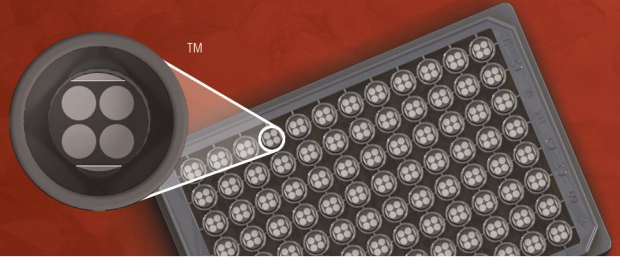
MSD® Phospho(Ser2448)/Total mTOR Assay Whole Cell Lysate Kit

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(Ser2448)/Total mTOR: Whole Cell Lysate Kit	
Kit size	
1 plate	K15170D-1
5 plates	K15170D-2
20 plates	K15170D-3

Phospho-mTOR (Ser2448) Whole Cell Lysate Set	
200 µg	C10JE-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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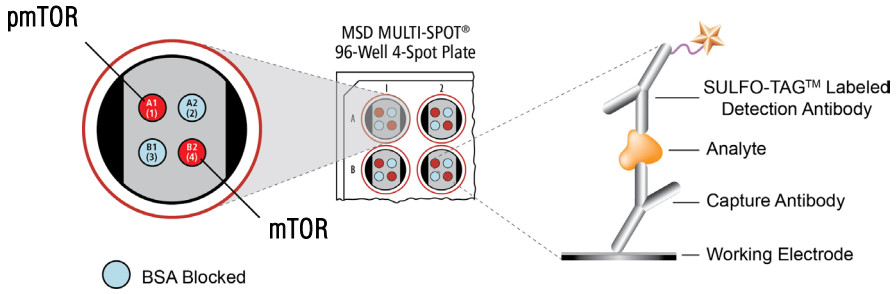

m-TOR (mammalian Target of Rapamycin or FRAP1) is a serine/threonine protein kinase that plays a role in cell growth, motility, proliferation, and survival as well as protein synthesis and transcription. m-TOR is a downstream signal regulator of receptors such as insulin, growth factors, and amino acids.[1] m-TOR is an important part of two larger protein complexes – mTORC1 and mTORC2. mTORC1 plays a critical role in nutrient and energy sensing as well as controlling protein synthesis. m-TOR, through its role as the active kinase within mTORC1 and mTORC2 plays a large role in the PI3K signaling cascade. Mutations in m-TOR, along with PI3K and Ras, frequently occur in cancers because of the regulatory roles these signaling molecules play in protein synthesis, cell cycle progression, and metabolism, as well as in controlling the transcription factors involved in regulation of these processes.[2] Inhibitors of m-TOR and regulators of the m-TOR pathway have been extensively studied and there are many efforts to develop pharmaceutical therapies to regulate this pathway in hopes of development of more effective cancer drugs. m-TOR inhibitors have been tested in clinical trials for treatment of breast cancer, non-small cell lung cancer, high grade gliomas, and multiple different solid tumors.[3]

The MSD Phospho(Ser2448)/Total mTOR Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Ser2448)/Total mTOR 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-mTOR (Ser2448) and total mTOR antibodies and are shown below for comparison. Growing HEK293 cells were treated with Wortmannin (100 nM, 3 hours) (negative) or PMA (1 µM, 30 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-mTOR antibody and anti-total mTOR antibody on spatially distinct electrodes within a well. Phosphorylated and total mTOR were detected with anti-total mTOR antibody conjugated with MSD SULFO-TAG™ reagent.

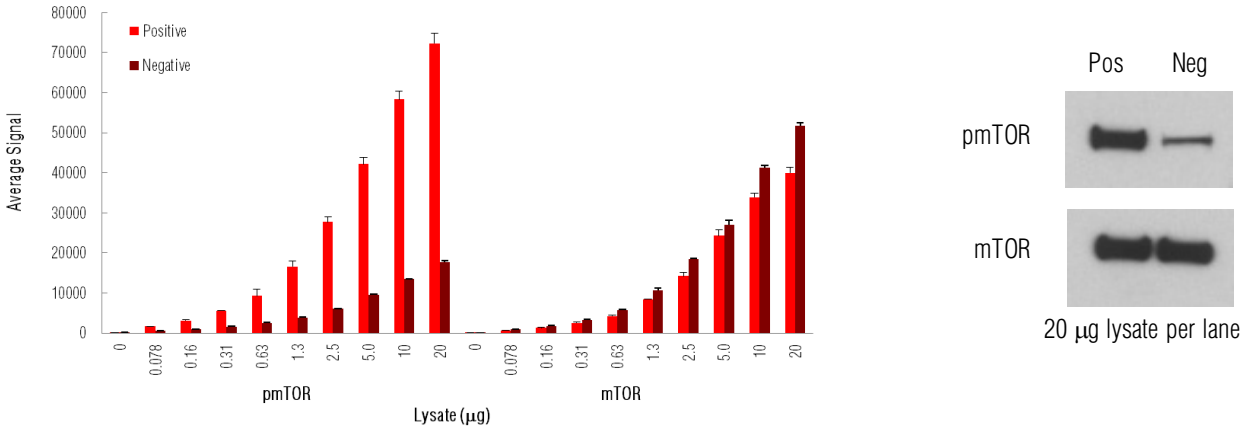

Fig. 1: Sample data generated with the MULTI-SPOT® Phospho(Ser2448)/Total mTOR Assay. Increased signal for phosphorylated mTOR was observed with pmTOR positive cell lysate. Total mTOR signal increased throughout the titration of both pmTOR positive and negative cell lysates. The Phospho(Ser2448)/Total mTOR Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pmTOR positive and negative HEK293 cell lysates using the MULTI-SPOT Phospho(Ser2448)/Total mTOR Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pmTOR	0	133	1	0.8	133	1	0.8	
	0.078	1674	21	1.2	624	25	4.1	2.7
	0.16	3086	197	6.4	990	74	7.4	3.1
	0.31	5594	0	0.0	1534	153	10.0	3.6
	0.63	9260	1708	18.4	2521	93	3.7	3.7
	1.3	16665	1308	7.8	3804	186	4.9	4.4
	2.5	27779	1317	4.7	6054	78	1.3	4.6
	5.0	42148	1699	4.0	9482	162	1.7	4.4
	10	58366	2047	3.5	13558	23	0.2	4.3
	20	72339	2546	3.5	17750	278	1.6	4.1
mTOR	0	84	2	2.5	84	2	2.5	
	0.078	698	33	4.8	897	1	0.1	0.8
	0.16	1330	68	5.1	1833	71	3.9	0.7
	0.31	2576	199	7.7	3311	59	1.8	0.8
	0.63	4262	319	7.5	5807	74	1.3	0.7
	1.3	8439	23	0.3	10767	390	3.6	0.8
	2.5	14244	838	5.9	18550	112	0.6	0.8
	5.0	24365	1341	5.5	27060	1184	4.4	0.9
	10	33878	1025	3.0	41300	508	1.2	0.8
	20	39963	1346	3.4	51708	869	1.7	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

For a complete list of products, please visit our website at www.mesoscale.com

References

1. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004 Aug 15;18(16):1926-45.
2. Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. ture. 2006 May 25;441(7092):424-30.
3. Markman B, Dienstmann R, Tabernero J. Targeting the PI3K/Akt/mTOR pathway--beyond rapalogs. Oncotarget. 2010 Nov;1(7):530-43.