## MSD<sup>®</sup> Phospho-mTOR (Ser2448) 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

Cytokines Hypoxia Immunogenicity Inflammation Metabolic Oncology Toxicology Vascular

Catalog Numbers

Phospho-mTOR (Ser2448)

Whole Cell Lysate Kit

Kit size

Phospho-mTOR (Ser2448)

Whole Cell Lysate Set

Ordering information

MSD Customer Service Phone: 1-301-947-2085

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K150JED-1

K150JED-2

K150JED-3

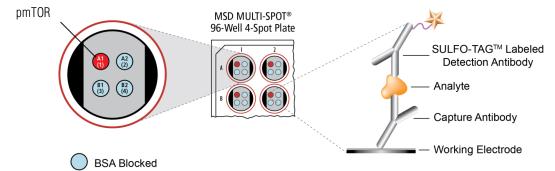
C10JE-1

1 plate

5 plates

20 plates

200 µg



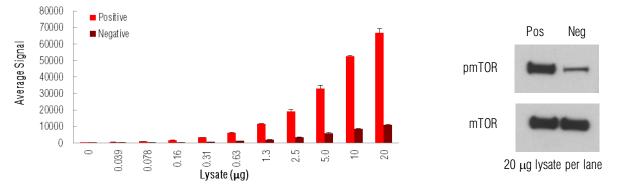
**m-TOR (mammalian Target of Rapamycin or FRAP1)** is a serine/threonine protein kinase that plays a role in cell growth, motility, proliferation, and survival as well as protein synthesis and transcription. m-TOR is a downstream signal regulator of receptors such as insulin, growth factors, and amino acids.<sup>1</sup> 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. Activated mTORC1 phosphorylates S6K1 and 4E-BP1.<sup>2,3</sup> mTORC2 is a cytoskeletal regulator and phosphorylates AKT.<sup>4,5</sup> 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.<sup>6</sup> 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.<sup>7</sup>

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

### Typical Data

# Representative results for the Phospho-mTOR (Ser2448) 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<sup>®</sup> 4-Spot plates coated with anti-total mTOR antibody on one of the four spatially distinct electrodes per well. Phosphorylated mTOR was detected with anti-phospho-mTOR antibody conjugated with MSD SULFO TAG<sup>™</sup> reagent.



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Fig. 1: Sample data generated with MULTI-ARRAY<sup>®</sup> Phospho-mTOR (Ser2448) Assay. Increased signal is observed with the titration of pmTOR positive cell lysate. The Phospho-mTOR (Ser2448) Assay provides a quantitative measure of the data obtained with the traditional Western blot.





#### Lysate Titration

Data for positive and negative HEK293 cell lysates using the MULTI-ARRAY Phospho-mTOR (Ser2448) are presented below.

Lysate	Positive			Negative			D/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	P/N
0	115	0	0.0	115	0	0.0	
0.039	557	15	2.7	209	6	2.7	2.7
0.078	939	25	2.6	290	6	2.2	3.2
0.16	1705	46	2.7	406	1	0.2	4.2
0.31	3338	55	1.7	713	1	0.2	4.7
0.63	6142	62	1.0	1195	35	2.9	5.1
1.3	11512	170	1.5	1991	6	0.3	5.8
2.5	19249	982	5.1	3389	45	1.3	5.7
5.0	32943	1894	5.8	5726	433	7.6	5.8
10	52675	324	0.6	8474	355	4.2	6.2
20	66628	2674	4.0	10908	296	2.7	6.1

#### 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

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