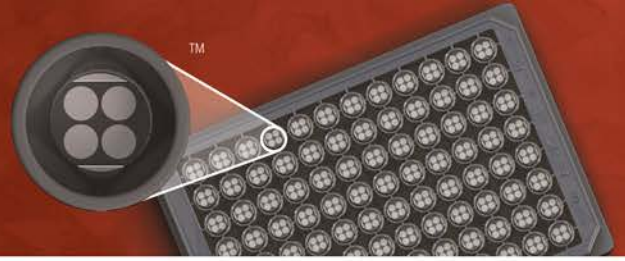
MSD® Phospho-4E-BP1 (Thr37/46) Assay Whole Cell Lysate Kit

For quantitative determination in human and mouse whole cell lysate samples



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

Catalog Numbers

Phospho-4E-BP1 (Thr37/46) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150KHD-1
5 plates	K150KHD-2
20 plates	K150KHD-3

Phospho-4E-BP1 Whole Cell Lysate Set	
200 µg	C10KH-1

Ordering information

MSD Customer Service
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Company Address

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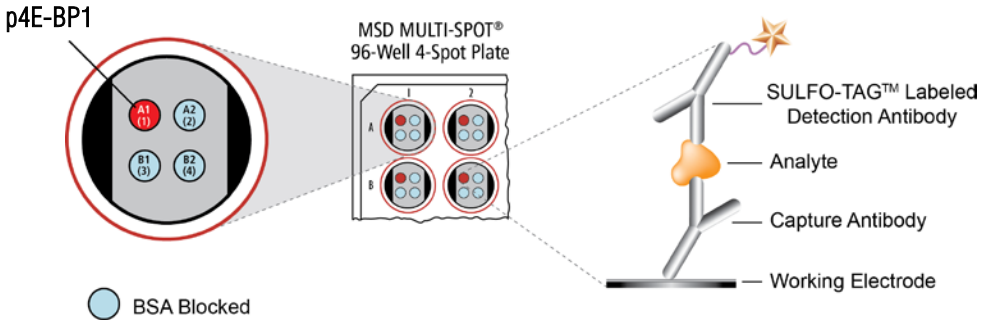
www.mesoscale.com®



The eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) is a translational repressor protein that plays a critical role in the control of protein synthesis, survival, and cell growth.[1] During cap-dependent translation, eIF4E binds to the mRNA cap structure and promotes formation of the eIF4F initiation complex and ribosome binding. Non-phosphorylated 4E-BP1 binds eIF4E and impedes formation of the initiation complex, blocking translation and favoring apoptosis.[1,2] However, when 4E-BP1 is phosphorylated, its affinity for eIF4E is reduced, allowing eIF4E to interact with the cap complex, and initiation ensues. 4E-BP1 has multiple phosphorylation sites and is most often phosphorylated through the mammalian target of rapamycin (mTOR) signaling pathway, although several other kinases have also been shown to phosphorylate this key repressor (cyclin-dependent kinase 1, P13K-Akt, and ERK1/2).[1-3] Phosphorylated 4E-BP1 expression in breast, ovary, and prostate tumors has been shown to be associated with tumor growth and malignant progression.[1,4] Thus, phosphorylated 4E-BP1 may prove a highly relevant biomarker in oncogenesis, and a better understanding of the signaling pathways utilizing this molecule may enhance the development of anti-cancer therapeutics and targets.

The MSD Phospho-4E-BP1 (Thr37/46) Assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-4E-BP1 (Thr37/46) 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-4E-BP1 (Thr37/46) and total 4E-BP1 antibodies and are shown for comparison.
Growing MCF-1 cells were treated with IGF-1 (100 nM; 20 minutes) (positive) or with LY294002 (50 µM; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-spot plates coated with anti-total 4E-BP1 antibody on one of the four spatially distinct electrodes within a well. Phosphorylated 4E-BP1 was detected with anti-phospho-4E-BP1 (Thr37/46) antibody conjugated with MSD SULFO-TAG™.

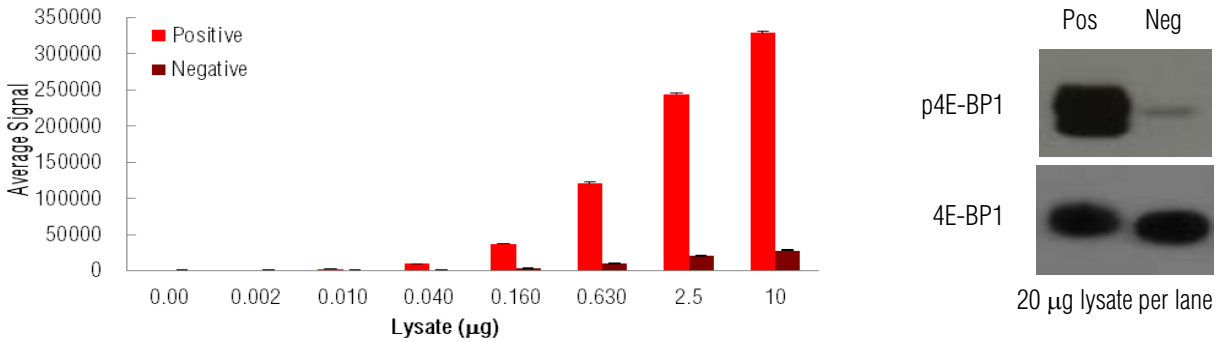


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-4E-BP1 (Thr37/46) Assay. Increased signal for phosphorylated 4E-BP1 was observed with p4E-BP1 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-4E-BP1 (Thr37/46) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for p4E-BP1 positive and negative MCF-7 cell lysates using the MULTI-ARRAY Phospho-4E-BP1 (Thr37/46) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	107	2	2.0	105	4	3.4	
0.0024	683	24	3.5	127	20	15.6	5.4
0.0098	2527	77	3.1	319	44	13.7	7.9
0.039	9766	235	2.4	854	41	4.8	11
0.16	37010	385	1.0	2925	18	0.6	13
0.63	120247	3022	2.5	9713	56	0.6	12
2.5	244122	1277	0.5	20818	412	2.0	12
10	328641	2727	0.8	27453	722	2.6	12

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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4. Pons B, Peg V, Vázquez-Sánchez MA, López-Vicente L, Argelaguet E, Coch L, Martínez A, Hernández-Losa J, Armengol G, Ramon Y Cajal S. The effect of p-4E-BP1 and p-eIF4E on cell proliferation in a breast cancer model. Int J Oncol. 2011 Nov;39(5):1337-45.