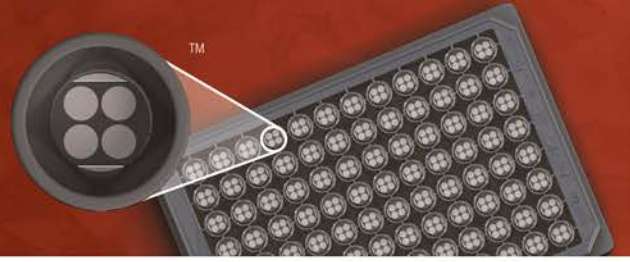
MSD® Phospho-FOXO3a (Thr32) Assay Whole Cell Lysate Kit

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-FOXO3a (Thr32) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150KKD-1
5 plates	K150KKD-2
20 plates	K150KKD-3

Phospho-FOXO3a Whole Cell Lysate Set	
200 µg	C10KK-1

Ordering information

MSD Customer Service
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Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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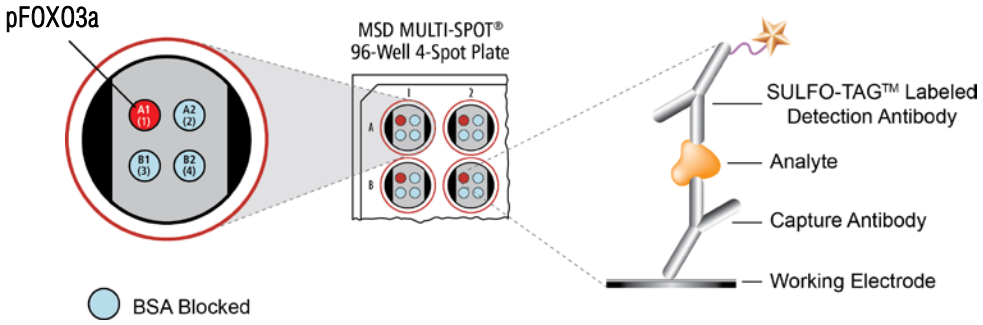
Not for use in diagnostic procedures.



Forkhead box O3a (FOXO3a) belongs to the O subclass of the forkhead family of transcription factors (FOXO1, FOXO3a, FOXO4, FOXO6) which are characterized by a forkhead DNA binding domain. This transcription factor regulates multiple transcriptional targets involved in various cellular processes, including proliferation, stress resistance, apoptosis, metabolism, and longevity.[1,2] As a transcription factor, FOXO3a plays a key role in cellular senescence through regulation of genes promoting cell cycle arrest and apoptosis. Increased proliferation results when FOXO3a is inactivated through phosphorylation by Akt or PI3K at Thr32, Ser253, and Ser315. This results in nuclear export and inhibition of transcription factor activity.[3] Other post-translational modifications, including acetylation, methylation, or interactions with reactive oxygen species (ROS), may also result in increased or abnormal FOXO3a activity.[1,2] Therefore, phosphorylation of FOXO3a is often associated with tumorigenesis and cancer development.[1] A better understanding of the regulation of FOXO3a activity and its specific transcriptional targets may provide strong insight into the mechanisms controlling cell fate decisions.

The MSD Phospho-FOXO3a (Thr32) Assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-FOXO3a (Thr32) 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-FOXO3a (Thr32) and total FOXO3a antibodies and are shown for comparison.
Growing MCF-7 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 FOXO3a antibody on one of the four spatially distinct electrodes within a well. Phosphorylated FOXO3a was detected with anti-phospho-FOXO3a (Thr32) antibody conjugated with MSD SULFO-TAG™.

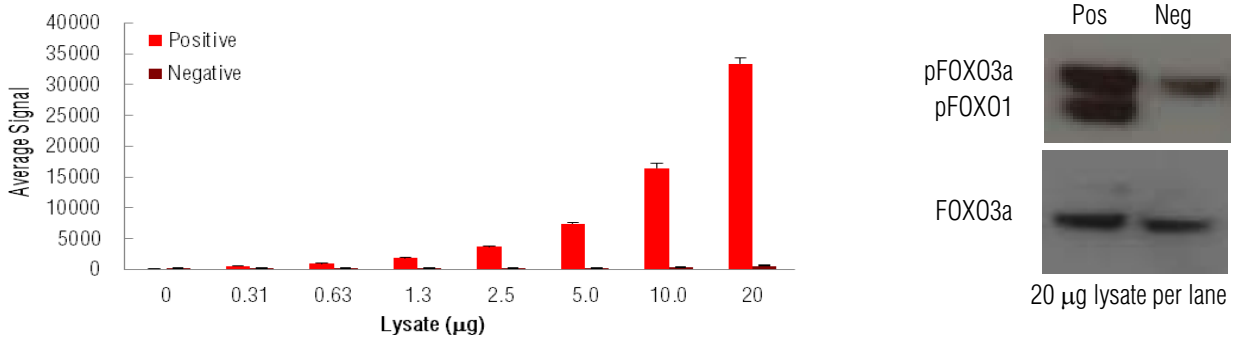


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-FOXO3a (Thr32) Assay. Increased signal for phosphorylated FOXO3a was observed with pFOXO3a positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-FOXO3a (Thr32) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pFOXO3a positive and negative MCF-7 cell lysates using the MULTI-ARRAY Phospho-FOXO3a (Thr32) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	159	7	4.4	163	1	0.4	
0.31	587	18	3.0	168	3	1.7	3.5
0.63	980	57	5.8	171	8	4.6	5.7
1.3	1850	66	3.6	191	9	4.8	10
2.5	3677	197	5.3	228	7	3.1	16
5.0	7431	255	3.4	271	17	6.3	27
10	16458	853	5.2	372	6	1.5	44
20	33328	1013	3.0	613	33	5.4	54

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. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. Oncogene. 2008 Apr 7;27(16):2312-9.
2. Miyamoto K, Miyamoto T, Kato R, Yoshimura A, Motoyama N, Suda T. FoxO3a regulates hematopoietic homeostasis through a negative feedback pathway in conditions of stress or aging. Blood. 2008 Dec 1;112(12):4485-93.
3. A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999 Mar 19;96(6):857-68.

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