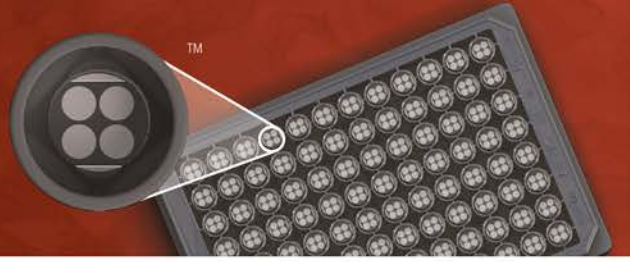
MSD® Phospho-BAD (Ser112) Assay Whole Cell Lysate Kit

For quantitative determination in human and monkey whole cell lysate samples



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

Catalog Numbers

Phospho-BAD (Ser112) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151CCD-1
5 plates	K151CCD-2
20 plates	K151CCD-3

Phospho-BAD Whole Cell Lysate Set	
200 µg	C11CC-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.
9238 Gaither Road
Gaithersburg, MD 20877 USA

www.mesoscale.com®

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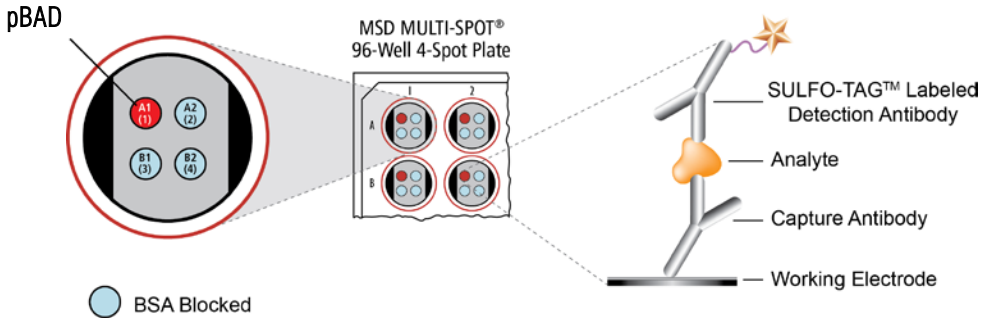

Bcl-2-antagonist of cell death protein (BAD), a member of the pro-apoptotic Bcl-2 family of proteins, functions by displacing the binding of Bax to Bcl-2 and Bcl xL, and causes cell death by apoptosis. The binding of cytokines and growth factors to cell surface receptors activates intracellular signal transduction cascades that promote cell survival. Akt phosphorylates BAD on Ser136. BAD is also phosphorylated by protein kinase A (PKA) and p90 ribosomal S6 kinase (p90RSK) on Ser112. Phosphorylated BAD binds to members of the 14-3-3 protein family. This inhibits its interaction with Bcl-2 and Bcl-xL through cytosolic sequestration. The anti-apoptotic proteins Bcl-2 and BcL-xL are then free to interact with Apaf-1 and BID, thus promoting cell survival.

The MSD Phospho-BAD (Ser112) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-BAD (Ser112) 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-BAD (Ser112) and total BAD antibodies and are shown below for comparison.
Serum deprived COS-7 cells were treated with staurosporine (1 µM, 3 hours) (negative), or treated with PMA (200 nM, 1 hour) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-BAD (Ser112) antibody on one of the four spatially distinct electrodes within a well. Phosphorylated BAD was detected with anti-total BAD antibody conjugated with MSD SULFO-TAG™ reagent.

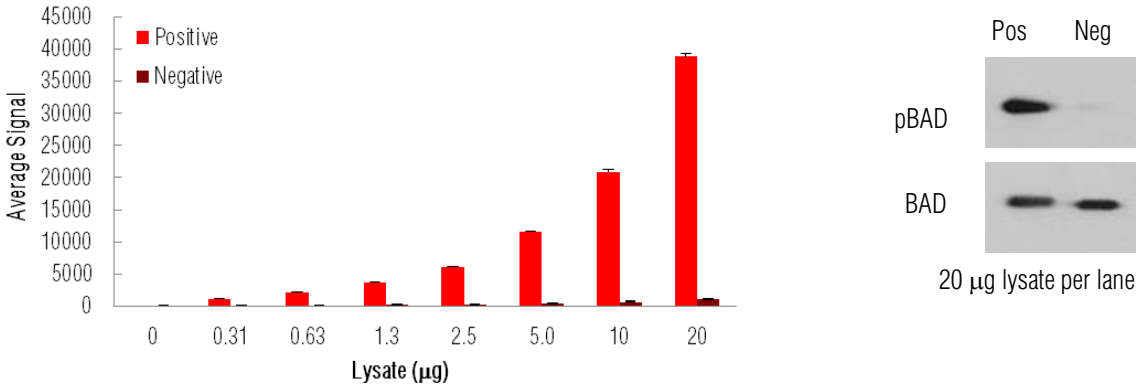

Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-BAD (Ser112) Assay. Increased signal for phosphorylated BAD was observed with only pBAD positive cell lysate. Signal for pBAD negative lysate remains low throughout the titration. The Phospho-BAD (Ser112) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pBAD positive and negative COS-7 cell lysates using the MULTI-ARRAY Phospho-BAD (Ser112) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	20	6	28.3	40	1	1.8	
0.31	1207	45	3.7	109	4	3.3	11
0.63	2108	115	5.4	156	6	4.1	14
1.3	3651	60	1.6	193	2	1.1	19
2.5	6125	133	2.2	282	4	1.5	22
5.0	11599	115	1.0	460	1	0.3	25
10	20851	393	1.9	692	6	0.8	30
20	38832	483	1.2	1085	18	1.6	36

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 using MSD's platform for the measurement of phosphoproteins

1. Carnahan J, Beltran PJ, Babij C, Le Q, Rose MJ, Vonderfecht S, Kim JL, Smith AL, Nagapudi K, Broome MA, Fernando M, Kha H, Belmontes B, Radinsky R, Kendall R, Burgess TL. Selective and potent Raf inhibitors paradoxically stimulate normal cell proliferation and tumor growth. Mol Cancer Ther. 2010 Aug;9(8):2399-410. Epub 2010 Jul 27.
2. Brake R, Starnes C, Lu J, Chen D, Yang S, Radinsky R, Borges L. Effects of palifermin on antitumor activity of chemotherapeutic and biological agents in human head and neck and colorectal carcinoma xenograft models. Mol Cancer Res. 2008 Aug;6(8):1337-46.
3. Gowan SM, Hardcastle A, Hallsworth AE, Valenti MR, Hunter LJ, de Haven Brandon AK, Garrett MD, Raynaud F, Workman P, Aherne W, Eccles SA. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol. 2007 Jun;5(3):391-401.