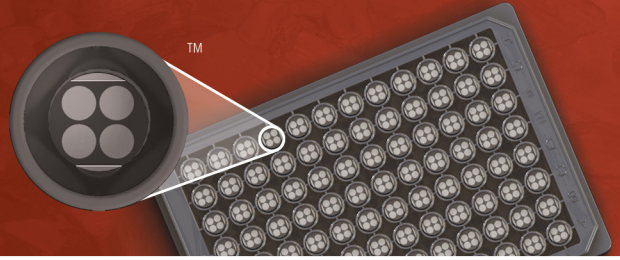
MSD® Total BAD Assay Whole Cell Lysate Kit

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

Total BAD Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151CDD-1
5 plates	K151CDD-2
20 plates	K151CDD-3

Phospho-BAD Whole Cell Lysate Set	
200 µg	C11CC-1

Ordering information

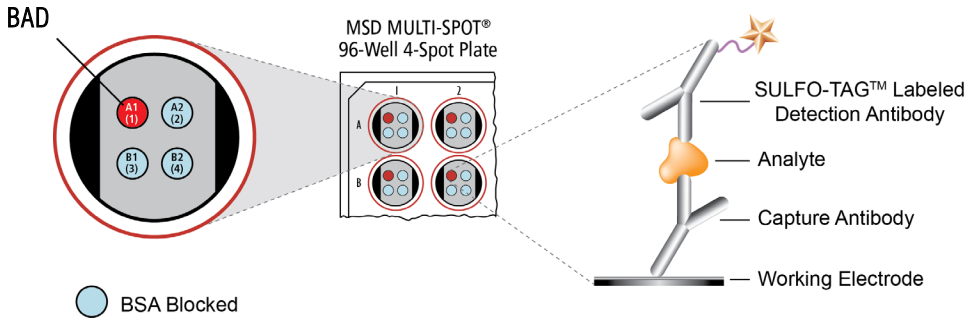
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA

www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures.



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 the family of 14-3-3 proteins. 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 Total BAD Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Total BAD 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-total BAD antibody on one of the four spatially distinct electrodes within a well. Total BAD was detected with anti-total BAD antibody conjugated with MSD SULFO-TAG™ reagent.

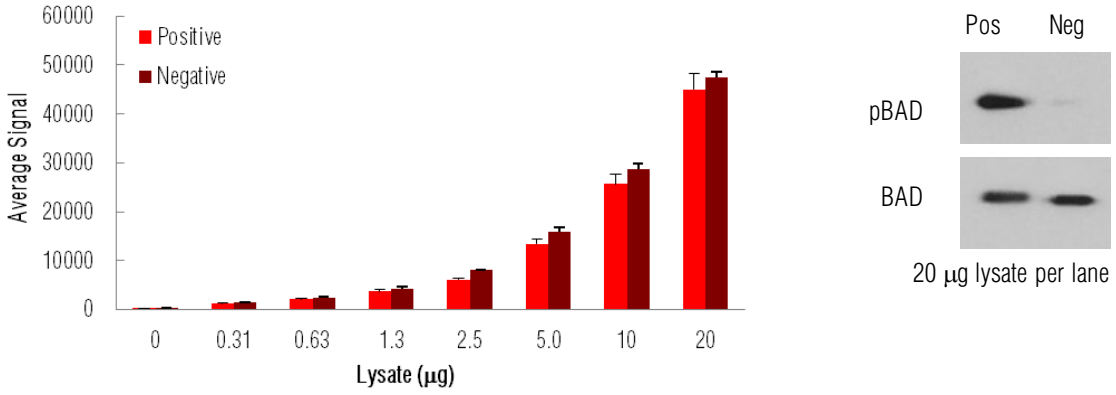


Fig. 1: Sample data generated with the MULTI-ARRAY® Total BAD Assay. Increased signal is observed with the titration of both pBAD positive and negative cell lysates. The Total BAD Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

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

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	274	23	8.5	302	3	0.9	
0.31	1234	198	16.0	1438	129	9.0	0.9
0.63	2079	206	9.9	2435	212	8.7	0.9
1.3	3726	395	10.6	4356	334	7.7	0.9
2.5	6151	367	6.0	8023	199	2.5	0.8
5.0	13420	1111	8.3	15857	1059	6.7	0.8
10	25645	1979	7.7	28664	1085	3.8	0.9
20	45019	3335	7.4	47574	1063	2.2	0.9

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.