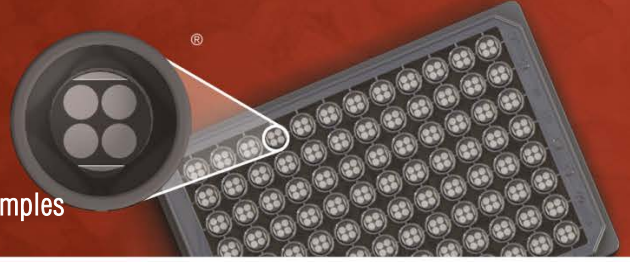
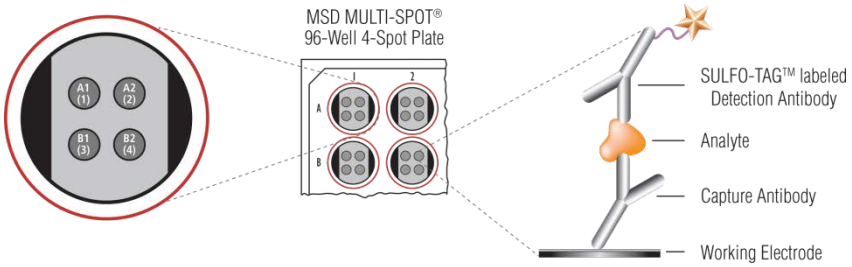
MSD® GAPDH Kit

For quantitative determination in human and non-human primate whole cell lysate samples



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

1. GAPDH
2. *BSA blocked*
3. *BSA blocked*
4. *BSA blocked*

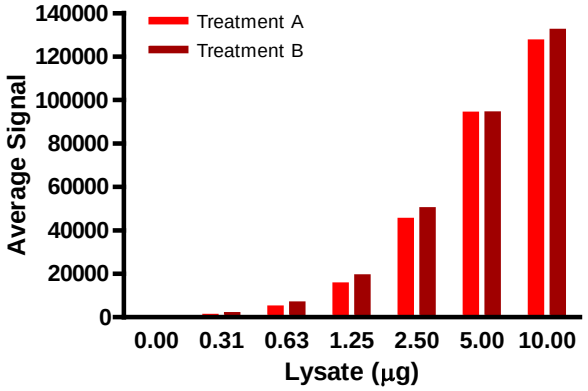


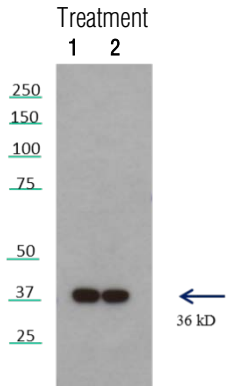
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is 37kDa enzyme that plays an important role in glycolysis, thus serving to break down glucose for energy and carbon molecules.[1] It is present in high levels in almost all tissues and is thought to be a ubiquitously expressed housekeeping gene.[2] GAPDH has also been described in non-metabolic processes, including DNA replication, RNA transport, transcription activation, apoptosis initiation, and the shuttling from the endoplasmic reticulum to the Golgi body.[3] GAPDH catalyzes the reversible, oxidative phosphorylation of glyceraldehyde 3-phosphate to D-glycerate 1,3-bisphosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD+) in two coupled steps. D-glycerate 1,3-bisphosphate is an important intermediate used for the synthesis of ATP.[1] GAPDH is reported to bind other proteins, including the amyloid precursor protein, which has been implicated in Alzheimer's disease; as well as the polyglutamine tracts of Huntingtin, which are causative of Huntington's disease.[4,5] The MSD GAPDH assay is available on 96-well, 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the GAPDH Kit are illustrated below. The signal and ratio values provided are examples; individual results will vary depending upon the samples tested. Western blot analyses of each lysate type are shown for comparison.

This assay was developed using cytoplasmic fraction of MCF7 cells treated with IGF-1 (treatment 1, 100 nM, 30 minutes) and LY294002 (treatment 2, 50 µM, 2.5 hours). Cytoplasmic cell lysates were added to MSD MULTI-SPOT®, 4-spot plates coated with anti-GAPDH antibody on one of the four spatially distinct electrodes in each well. GAPDH was detected with anti-GAPDH antibody conjugated with MSD SULFO-TAG™ reagent.





10 µg lysate per lane

Figure 1: Sample data generated with GAPDH assay. Increased signal is observed with the titration of treated lysates. The GAPDH assay provides a quantitative measure of the data obtained with the traditional Western blot.

Catalog Numbers

GAPDH Kit	
Kit Size	Catalog #
1 plate	K151PWD-1
5 plates	K151PWD-2
25 plates	K151PWD-4

Ordering Information

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

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Rockville, MD 20850-3173 USA
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Sample Titration

Data for treated cell lysates using the GAPDH Kit are presented below.

Lysate	Treatment 1			Treatment 2		
(µg/well)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV
0	64	15	23.4	95	14	14.9
0.31	2666	56	2.1	3476	197	5.7
0.63	6500	197	3.0	8307	281	3.4
1.3	17 076	470	2.8	20 822	249	1.2
2.5	46 857	1602	3.4	51 701	457	0.9
5.0	95 685	808	0.8	95 830	443	0.5
10	128 982	4372	3.4	133 853	1016	0.8

For a complete list of products, please visit our website at www.mesoscale.com.

The MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample volumes of 25 µL 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 response (light signal), minimizing matrix interference
- **Simple protocols:** Only labels bound near the electrode surface are excited, enabling assays with fewer washes
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules

References

1. Sirover M. On the functional diversity of glyceraldehyde-3-phosphate dehydrogenase: biochemical mechanisms and regulatory control. Biochim Biophys Acta. 2011 Aug;1810(8):741-51
2. Barber R, et al. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics. 2005 May 11;21(3):389-95.
3. Tristan C, et al. The diverse functions of GAPDH: views from different subcellular compartments. Cell Signal. 2011 Feb;23(2):317-23.
4. Wang Q, et al. Proteomic analysis of neurofibrillary tangles in Alzheimer disease identifies GAPDH as a detergent-insoluble paired helical filament tau binding protein. FASEB J. 2005 May;19(7):869-71.
5. Bae B, et al. Mutant huntingtin: nuclear translocation and cytotoxicity mediated by GAPDH. Proc Natl Acad Sci U S A. 2006 Feb 28;103(9):3405-9.