MSD[®] Phospho-Met (Tyr1349) Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples

pMet



Alzheimer's Disease BioProcess Cardiac Cell Signaling Clinical Immunology Cytokines

Cytokines Hypoxia Immunogenicity Inflammation Metabolic Oncology Toxicology Vascular

Catalog Numbers

Phospho-Met (Tyr1349)

Assay: Whole Cell Lysate Kit

Kit size

SULFO-TAG[™] Labeled Detection Antibody Analyte Capture Antibody BSA Blocked

c-Met, also known as the hepatocyte growth factor receptor, is a proto-oncogene with tyrosine kinase activity. Hepatocyte growth factor (HGF) is the only identified ligand for Met and upon ligand binding, the Met receptor dimerizes, autophosphorylates its catalytic residues, and prepares to bind adaptor proteins to continue signaling through downstream mediators.¹ HGF activation of Met induces growth, proliferation, cell survival, motility, and angiogenesis.² Met has a tyrosine kinase domain, containing phosphorylated residues Tyr1234 and Tyr1235, and a multi-substrate docking site, containing phosphorylated residues Tyr1349 and Tyr1356.³ Phosphorylation of these residues are key to the direct biological effects of Met activation as well as the ability of Met to signal through other downstream signaling cascades such as the PI3K signaling cascade, SRC, STAT, and Ras-Raf-Mek-Erk cascades.⁴ Due to the involvement of Met in different types of cancers, there has been a lot of research and drug development directed towards disruption and modulation of the HGF-Met interactions and the downstream signaling cascades controlled through Met-HGF binding.⁴

The MSD Phospho-Met (Tyr1349) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-Met (Tyr1349) 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-Met (Tyr1349) and total Met antibodies and are shown below for comparison.

Growing HeLa cells (negative) were treated with sodium vanadate (1 mM; 4 hours) and HGF (200 ng/mL; 5 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT[®] 4-Spot plates coated with anti-phospho-Met antibody on one of the four spatially distinct electrodes per well. Phosphorylated Met was detected with anti-total Met antibody conjugated with MSD SULFO-TAG[™] reagent.

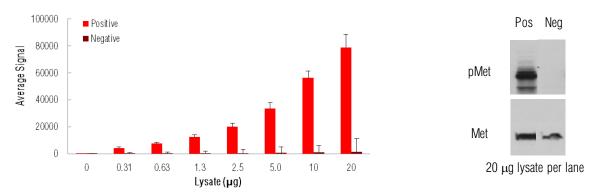


Fig. 1: Sample data generated with the MULTI-ARRAY[®] Phospho-Met (Tyr1349) Assay. Increased signal is observed with the titration of pMet positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-Met (Tyr1349) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

 1 plate
 K151DLD-1

 5 plates
 K151DLD-2

 20 plates
 K151DLD-3

 Phospho-Met (Tyr1349)

	Phospho-Met (Tyr1349) Whole Cell Lysate Set				
200 μ g	C11DL-1				

Ordering information

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

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Lysate Titration

Data for pMet positive and negative HeLa cell lysates using the MULTI-ARRAY Phospho-Met (Tyr1349) Assay are presented below.

Lysate	Positive			Negative			P/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	E / N
0	49	16	32.8	65	16	23.8	
0.31	4234	733	17.3	223	28	12.6	19
0.63	7499	939	12.5	273	40	14.8	27
1.3	12624	1574	12.5	423	65	15.3	30
2.5	20002	2703	13.5	513	108	21.0	39
5.0	33434	4394	13.1	681	49	7.1	49
10	56184	5092	9.1	1022	76	7.4	55
20	78824	9692	12.3	1462	225	15.4	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

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

References

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- 2. Eder JP, Vande Woude GF, Boerner SA, LoRusso PM. Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. Clin Cancer Res. 2009 Apr 1;15(7):2207-14.
- 3. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol. 2003 Dec;4(12):915-25.
- 4. Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov. 2008 Jun;7(6):504-16.

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