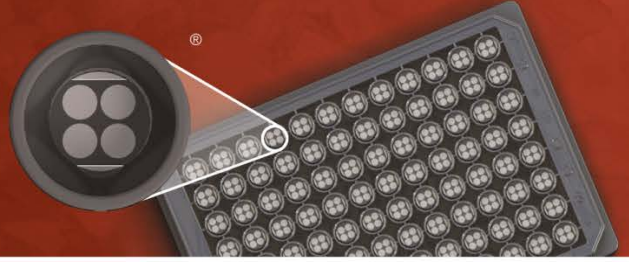
MSD® Total IRE-1α Kit

For quantitative determination in human, mouse, and rat whole cell lysate samples



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

Catalog Numbers

Total IRE-1α Kit	
Kit Size	Catalog #
1 plate	K153OHD-1
5 plates	K153OHD-2
25 plates	K153OHD-4

Ordering Information

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

Scientific Support

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Email: ScientificSupport@mesoscale.com

Company Address

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A division of
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Rockville, MD 20850-3173 USA
www.mesoscale.com®

1. IRE-1α
2. *BSA blocked*
3. *BSA blocked*
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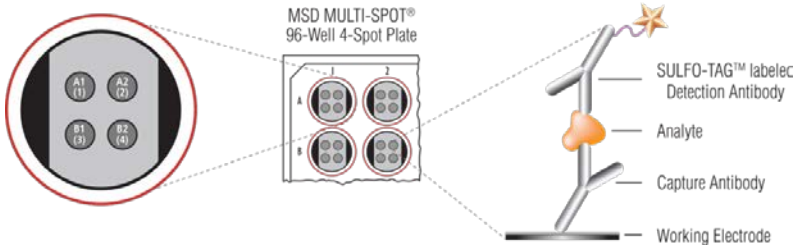
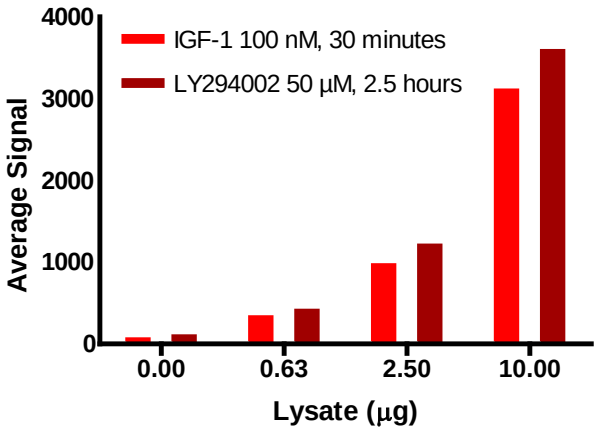


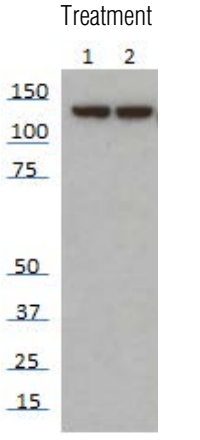
Inositol-requiring enzyme-1α (IRE-1α) is an endoplasmic reticulum (ER) transmembrane protein that mediates one of the three arms of unfolded protein response (UPR) adaptation to ER stressors. The ER is the predominant subcellular compartment for calcium storage, lipid production, and protein biosynthesis. Extracellular signaling molecules and protein receptors critical for cellular homeostasis are folded, assembled, matured, and secreted by the ER. However, ER stressors can overwhelm folding activity and unfolded proteins can accumulate in the ER, activating UPR pathways. In response to recognition of misfolded proteins in the ER lumen, IRE-1α oligomerizes to activate its cytoplasmic kinase and endoribonuclease domains, which initiate cleavage of the mRNA encoding the transcription factor XBP-1 (x-box binding protein 1), an effector that drives the transcription of cytoprotective genes.[1] IRE-1α can either return to an inactive state upon resolution of ER stress or enter a refractive state in which it no longer responds to unresolved ER stress after prolonged activation, limiting the cytoprotective duration of the UPR.[2] Increasing evidence suggests that ER stress and IRE-1α are associated with adverse conditions such as diabetes, cancer, muscle degeneration, and neurodegenerative, bipolar, liver, cardiac, and autoimmune diseases.[3-5] The MSD Total IRE-1α assay is available on 96-well, 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Total IRE-1α 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.

Growing MCF-7 cells were treated with 100 nM IGF-1 for 20 minutes (treatment 1) or with 50 µM LY294002 for 2.5 hours (treatment 2). Cell lysates were added to MSD MULTI-SPOT®, 4-spot plates coated with anti-total IRE-1α antibody on one of the four spatially distinct electrodes in each well. Total IRE-1α was detected with anti-total IRE-1α antibody conjugated with MSD SULFO-TAG™ reagent.





10 µg lysate per lane

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

Figure 1: Sample data generated with Total IRE-1α assay. Increased signal is observed with the titration of lysates treated with 100 nM IGF-1 for 20 minutes (treatment 1) and with lysates treated with 50 µM LY294002 for 2.5 hours (treatment 2). The Total IRE-1α assay provides a quantitative measure of the data obtained with the traditional Western blot.

Sample Titration

Data for positive and negative cell lysates using the Total IRE-1α Kit are presented below.

Lysate	Treatment 1			Treatment 2		
(µg/well)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV
0	110	13	12.1	148	33	22.4
0.63	383	20	5.2	462	22	4.9
2.5	1018	18	1.7	1257	59	4.7
10	3155	100	3.2	3639	135	3.7

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. Yoshida H, et al. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell. 2001 Dec 28;107(7):881-91.
2. Li H, et al. Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. PNAS. 2010 Sep 14;107(37):16113–8.
3. Wang S, et al. The impact of the unfolded protein response on human disease. J Cell Biol. 2012 Jun 25;197(7):857-67. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012 Jan 18;13(2):89-102.
4. Rath E, Haller D. Inflammation and cellular stress: a mechanistic link between immune-mediated and metabolically driven pathologies. Eur J Nutr. 2011 Jun;50(4):219-33.
5. Ozcan U, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004 Oct 15;306(5695):457-61.