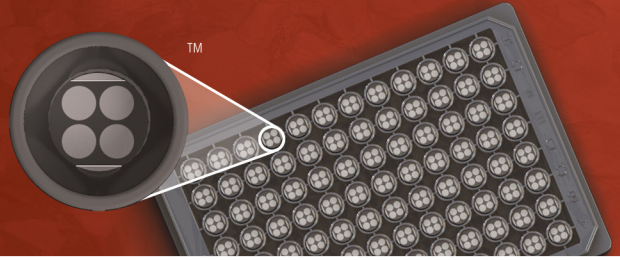
MSD® Phospho(Ser608)/Total Rb Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples



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

Catalog Numbers

Phospho (Ser608)/Total Rb: Whole Cell Lysate Kit	
Kit size	
1 plate	K15166D-1
5 plates	K15166D-2
20 plates	K15166D-3

Ordering information

MSD Customer Service
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Email: CustomerService@mesoscale.com

Company Address

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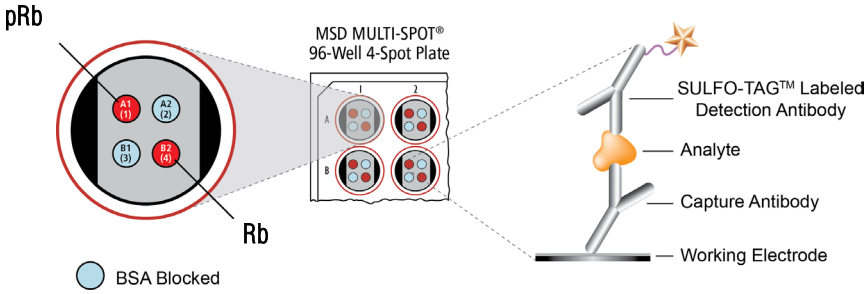
For Research Use Only. Not for use in diagnostic procedures.



The **retinoblastoma** protein (**Rb**, pRb, and Rb1) is a 110 kDa tumor suppressor protein (and a member of the pocket protein family) that functions by inhibiting progression from G1 to S phase of the cell cycle.[1] Rb is also involved in terminal differentiation and apoptosis.[2] It binds and inhibits transcriptional activity of members of the E2F family of transcription factors.[3] When Rb is phosphorylated by members of the Cyclin Dependent Kinase family (CDKs), it loses its affinity for the E2F transcription factors, transcriptional repression is relieved, and the cells proceed through the G1 to S phase transition and go on with the cell cycle.[4] Activation of the Cyclin D-dependent kinases can be prevented by Inhibitor of Kinase 4 (INK4), as well as by other mechanisms. Overexpression of INK4 inhibits phosphorylation of Rb by CDKs, and thus prevents cell cycle progression.[5] Loss of transcriptional repression by Rb is involved in many different types of cancer. Study of the retinoblastoma protein and inactivation of Rb by phosphorylation at multiple different residues has been the subject of intense study due to the fundamental role of Rb in many normal and disease based physiological processes.

The MSD Phospho(Ser608)/Total Rb Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Ser608)/Total Rb 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-Rb (Ser608) and total Rb antibodies and are shown below for comparison.
Growing HT29 cells were treated with tetrandrine (30 µM, 18 hours) and harvested 8 hours after a feed with complete medium (negative), or treated with nocodazole (0.2 µg/mL, 18 hours) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phopsho-Rb and anti-total Rb antibodies on spatially distinct electrodes within a well. Phosphorylated and total Rb were detected with anti-total Rb antibody conjugated with MSD SULFO-TAG™ reagent.

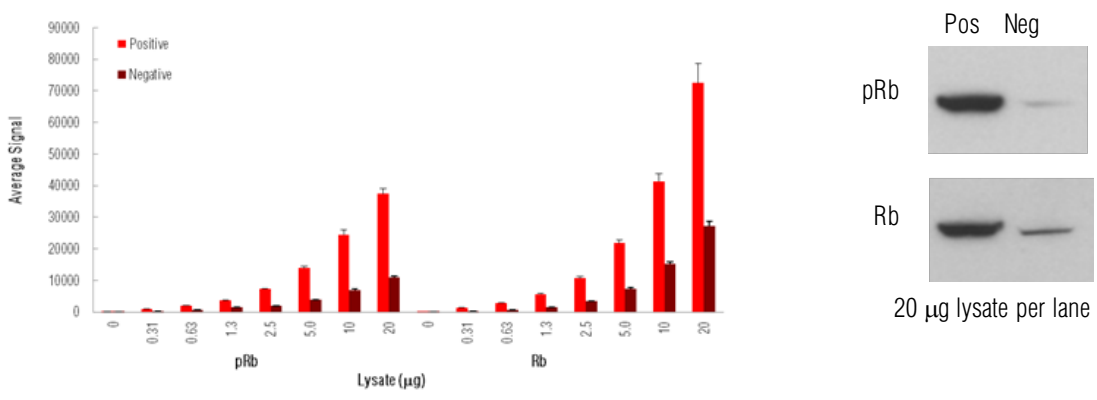


Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Ser608)/Total Rb Assay. The Phospho(Ser608)/Total Rb Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pRb positive and negative HT29 cell lysates using the MULTI-SPOT Phospho(Ser608)/Total Rb Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pRb	0	88	14	16.0	69	17	25.2	
	0.31	906	52	5.7	309	34	11.0	2.9
	0.63	2015	49	2.4	602	48	8.0	3.3
	1.3	3643	128	3.5	1531	44	2.9	2.4
	2.5	7273	151	2.1	2052	16	0.8	3.5
	5.0	13840	540	3.9	3856	89	2.3	3.6
	10	24484	1464	6.0	6830	405	5.9	3.6
	20	37451	1569	4.2	10961	346	3.2	3.4
Rb	0	14	16	108.6	26	13	47.8	
	0.31	1419	102	7.2	335	6	1.6	4.2
	0.63	2855	94	3.3	730	25	3.4	3.9
	1.3	5626	205	3.6	1586	52	3.3	3.5
	2.5	10665	542	5.1	3381	127	3.8	3.2
	5.0	21817	1061	4.9	7372	385	5.2	3.0
	10	41383	2465	6.0	15132	691	4.6	2.7
	20	72514	6125	8.4	27118	1471	5.4	2.7

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. Poznic M. Retinoblastoma protein: a central processing unit. J. Biosci. 2009 Jun;34(2):305-12.
3. Weintraub SJ, Chow KN, Luo RX, Zhang SH, He S, Dean DC. Mechanism of active transcriptional repression by the retinoblastoma protein. Nature. 1995 jun 29;375(6534):812-5.
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5. Hirai H, Roussel MF, Kato JY, Ashmun RA and Sherr CJ. Novel INK4 proteins, p19 and p18, are specifi c inhibitors of the cyclin D-dependent kinases