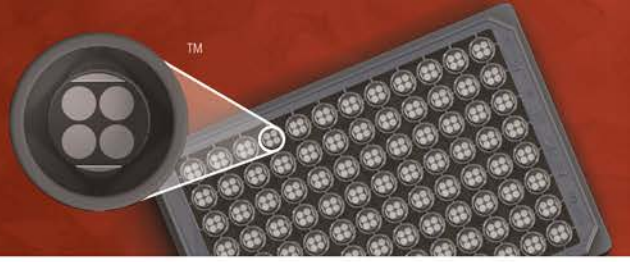
MSD® Total Rb Assay
Whole Cell Lysate Kit

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

Total Rb Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150IRD-1
5 plates	K150IRD-2
20 plates	K150IRD-3

Ordering information

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

Company Address

MESO SCALE DISCOVERY®
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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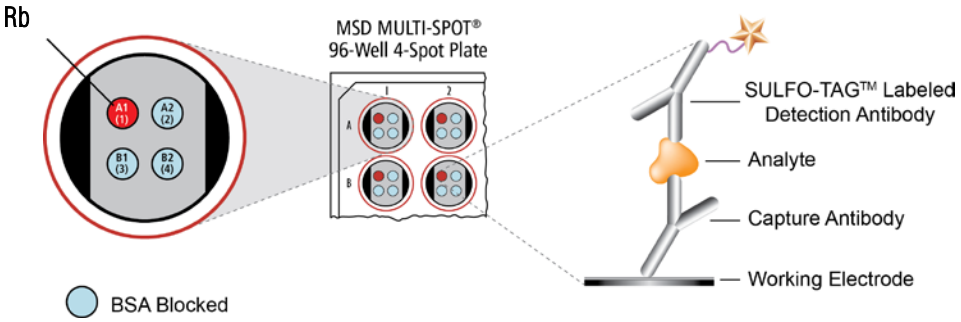


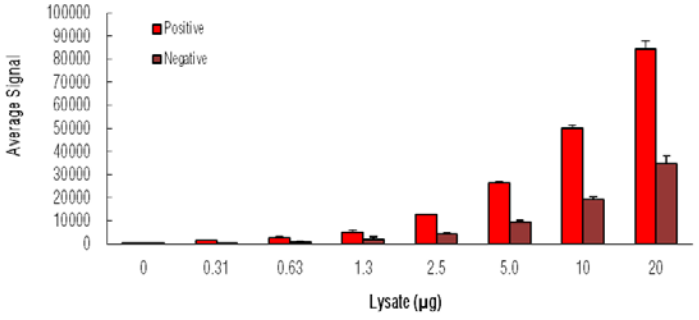
The **retinoblastoma** protein (**Rb**, pRb, and Rb1) is a 110 kDa tumor suppressor protein (and a member of the pocket protein family) that functions by inhibiting progression from G1 to S phase of the cell cycle.[1] Rb is also involved in terminal differentiation and apoptosis.[2] It binds and inhibits 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.

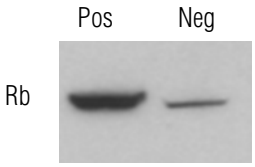
The MSD Total Rb Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the 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 analysis was performed with total Rb antibody and is shown below.

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





20 µg lysate per lane

Fig. 1: Sample data generated with the MULTI-ARRAY® Total Rb Assay. Increased signal is observed with the titration of both pRb positive and pRb negative cell lysates. The 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-ARRAY Total Rb Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	32	6	19.0	29	6	19.5	
0.31	1378	103	7.5	413	11	2.7	3.3
0.63	2671	405	15.1	962	84	8.7	2.8
1.3	4958	961	19.4	1955	234	12.0	2.5
2.5	12939	205	1.6	4181	209	5.0	3.1
5.0	26282	751	2.9	9417	685	7.3	2.8
10	50191	1203	2.4	19135	1810	9.5	2.6
20	84210	3486	4.1	34684	4124	11.9	2.4

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

1. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995 May 5;81(3):323-30.
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.
4. Knudsen ES, Wang JY. Differential regulation of retinoblastoma protein function by specific Cdk phosphorylation sites. J Biol Chem. 1996 Apr 5;271(14):8313-20.
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 CDK4 and CDK6. Mol Cell Biol. 1995 May;15(5):2672-81.