

Phospho-Rb (Ser608) Monoclonal Antibody (51B7)

Catalog Number **MA5-12584**

Product data sheet

Details		Species Reactivity	
Size	500 µL	Species reactivity	Human, Mouse
Host/Isotope	Mouse / IgG1	Published species	Rat, Mouse, Human, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	Immunocytochemistry (ICC)	Dilution * 1-2 µg/mL
Clone	51B7	Immunofluorescence (IF)	1-2 µg/mL
Conjugate	Unconjugated	Western Blot (WB)	1-2 µg/mL
Form	Liquid	Published Applications	
Concentration	0.2 mg/mL	Western Blot (WB)	See 1 publications below
Purification	Protein G	Immunohistochemistry (IHC)	See 3 publications below
Storage buffer	PBS, pH 7.4, with 0.2% BSA	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Contains	0.09% sodium azide		
Storage Conditions	4° C		

Product specific information

This antibody was originally validated as part of a Thermo Scientific Cellomics High Content Screening Kit. The antibody sold separately may have slightly different performance and may need to be further optimized for the best results.

Background/Target Information

Rb is a tumor suppressor nuclear phosphoprotein capable of binding to DNA. It is phosphorylated on serine and threonine, but not on tyrosine residues. It forms a complex with SV40 large T antigen, adenovirus E1A, and human papilloma virus-16 E. Rb protein may act by regulating transcription and loss of its function leads to uncontrolled cell growth. Aberrations in the RB gene have been implicated in cancers of breast, colon, prostate, kidney, nasopharynx, and leukemia.

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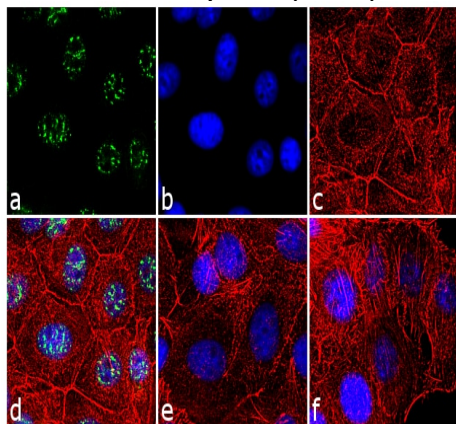
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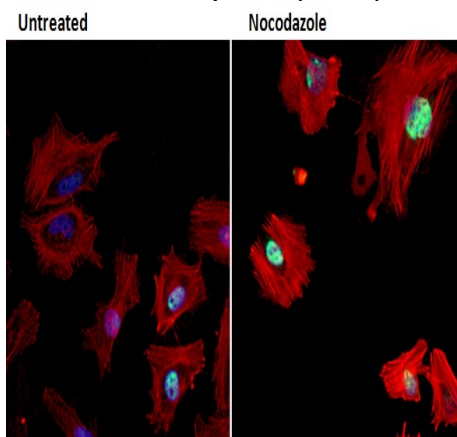
Advanced Verification Data

Phospho-Rb (Ser608) Antibody (MA5-12584)



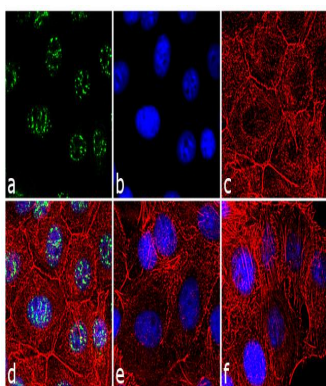
Detection of altered subcellular localization of the target protein by cell treatment demonstrates antibody specificity. Immunofluorescence analysis of Phospho-Rb (Ser608) using Phospho-Rb (Ser608) Monoclonal Antibody (Product # MA5-12584), shows localization of Phospho-Rb (Ser608) in the nucleus of COLO 205 cells upon being serum starved for 16 hrs. [Cell treatment validation info.](#)

Phospho-Rb (Ser608) Antibody (MA5-12584)



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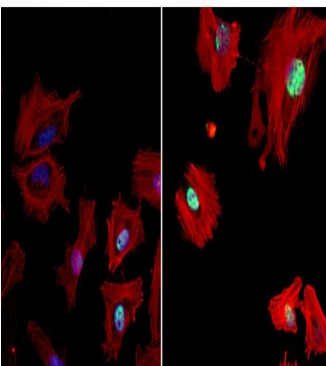
Product Images For Phospho-Rb (Ser608) Monoclonal Antibody (51B7)



Phospho-Rb (Ser608) Antibody (MA5-12584) in IF

Immunofluorescence analysis of Phospho-Retinoblastoma pSer608 was done on 70% confluent log phase Colo cells treated with serum starvation for 16 hours. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Phospho-Retinoblastoma pSer608 Antibody (51B7) Mouse Monoclonal Antibody (Product # MA5-12584) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d is a merged image showing punctate nuclear localization. Panel e is untreated cell with no signal. Panel f is a no primary antibody control. The images were captured at 60X magnification.

Untreated Nocodazole



Phospho-Rb (Ser608) Antibody (MA5-12584) in IF

Immunofluorescent analysis of Phospho-Retinoblastoma (Phospho-Rb) pSer608 (green) in HeLa cells either left untreated (left panel) or treated with 50nM Nocodazole (right panel) for 16 hours. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA (Product # 37525) for 15 minutes at room temperature. Cells were probed with a Phospho-Rb pSer608 monoclonal antibody (Product # MA5-12584) at a dilution of 1:100 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. F-Actin (red) was stained with DyLight 554 Phalloidin (Product # 21834) and nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan and ToxInsight Instrument at 20X magnification.

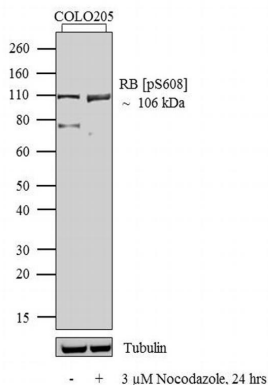
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Phospho-Rb (Ser608) Antibody (MA5-12584) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of COLO205 (Lane 1) and COLO205 treated with 3 µM of Nocodazole for 24 hrs (Lane 2). The blots were probed with Anti-Rb (pS608) Mouse Monoclonal Antibody (Product # MA5-12584, 1-2 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (Product # 62-6520, 1:4000 dilution). A 106 kDa corresponding to RB (pS608) was observed across the cell lines tested and was enhanced upon treatment. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



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PubMed References For Phospho-Rb (Ser608) Monoclonal Antibody (51B7)

1 Western Blot References

Species / Dilution	Summary
Human / Not Cited	<p>MA5-12584 was used in western blot to investigate the influence of selective inhibition of rRNA transcription on the cell cycle of human cancer cells</p> <p>Journal of cell science (Sep 2011; 124: 3017) "Selective inhibition of rRNA transcription downregulates E2F-1: a new p53-independent mechanism linking cell growth to cell proliferation." Author(s):Donati G,Brighenti E,Vici M,Mazzini G,Treré D,Montanaro L,Derezini M PubMed Article URL:http://dx.doi.org/10.1242/jcs.086074</p>

3 Immunohistochemistry References

Species / Dilution	Summary
Human / Not Cited	<p>MA5-12584 was used in immunohistochemistry to develop a novel HPV-16-positive head and neck carcinoma cell line containing a significant proportion of cancer stem cells</p> <p>Head and neck (Oct 2012; 34: 1480) "UM-SCC-104: a new human papillomavirus-16-positive cancer stem cell-containing head and neck squamous cell carcinoma cell line." Author(s):Tang AL,Hauff SJ,Owen JH,Graham MP,Czerwinski MJ,Park JJ,Walline H,Papagerakis S,Stoerker J,McHugh JB,Chepeha DB,Bradford CR,Carey TE,Prince ME PubMed Article URL:http://dx.doi.org/10.1002/hed.21962</p>
Rat / Not Cited	<p>MA5-12584 was used in immunohistochemistry to study the role of undifferentiated epithelial cells in the proliferation capacity of the renal proximal tubule</p> <p>American journal of physiology. Cell physiology (Jan 2008; 294: C22) "Proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells." Author(s):Vogetseder A,Picard N,Gaspert A,Walch M,Kaissling B,Le Hir M PubMed Article URL:http://dx.doi.org/10.1152/ajpcell.00227.2007</p>
Mouse / 1:40	<p>MA5-12584 was used in immunohistochemistry to study the role of cellular senescence in the beneficial effects of bexarotene in a murine mammary carcinogenesis model</p> <p>Cancer prevention research (Philadelphia, Pa.) (Apr 2013; 6: 299) "Bexarotene induces cellular senescence in MMTV-Neu mouse model of mammary carcinogenesis." Author(s):Shilkaitis A,Bratescu L,Green A,Yamada T,Christov K PubMed Article URL:http://dx.doi.org/10.1158/1940-6207.CAPR-12-0260</p>

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