

RICTOR Monoclonal Antibody (J.644.6)

Catalog Number      MA5-15006

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human, Mouse, Non-human primate, Rat
Host/Isotope	Rabbit / IgG		
Class	Monoclonal	Tested Applications	
Type	Antibody	Western Blot (WB)	Dilution * 1:1,000
Clone	J.644.6	* 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.	
Immunogen	Synthetic peptide corresponding to the sequence Gln1681 of human Rictor		
Conjugate	Unconjugated		
Form	Liquid		
Concentration	172 µg/mL		
Purification	Affinity chromatography		
Storage buffer	0.01M HEPES, pH 7.5, with 0.15M NaCl, 100µg/mL BSA, 50% glycerol		
Contains	<0.02% sodium azide		
Storage Conditions	-20°C		

Product specific information

It is not recommended to aliquot this antibody.

Background/Target Information

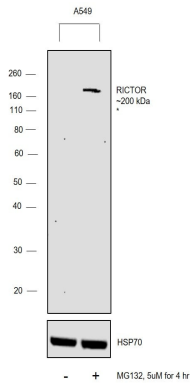
RICTOR and MTOR (FRAP1) are components of a protein complex that integrates nutrient- and growth factor-derived signals to regulate cell growth.

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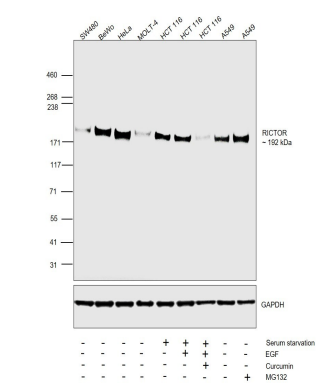
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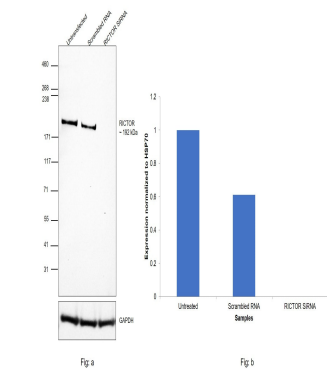
RICTOR Antibody (MA5-15006)

Altered expression of proteins upon cell treatment demonstrates antibody specificity. Western blot using RICTOR Monoclonal Antibody (J.644.6) (Product # MA5-15006), shows expression of RICTOR in A549 cells treated with MG132 (5  $\mu$ M for 4hr). {TM}



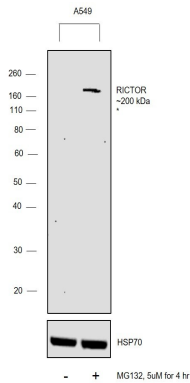
RICTOR Antibody (MA5-15006) in WB

Western blot was performed using Anti-RICTOR Polyclonal Antibody (Product # MA5-15006) and a 192 kDa band corresponding to RICTOR was observed across cell lines. Whole cell extracts (40  $\mu$ g lysate) of SW480 (Lane 1), BeWo (Lane 2), HeLa (Lane 3), MOLT4 (Lane 4), HCT 116 serum starved overnight (Lane 5), HCT116 serum starved overnight and treated with EGF (20 ng/mL for 45 mins) (Lane 6), HCT116 serum starved overnight, treated with Curcumin 25  $\mu$ M for 2 hrs and then treated with EGF (20 ng/mL for 45 mins) (Lane 7), A549 (Lane 8), A549 treated with MG132 5  $\mu$ M for 3 hrs (Lane 9) were electrophoresed using NuPAGE™ 3-8% Tris-Acetate Protein Gel (Product # EA0378BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (\*1:1000) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20000 dilution) using the iBright™ FL1500 Imaging System (Product # A44115). Chemiluminescent detection was performed using SuperSignal™ West Atto Ultimate Sensitivity Substrate (Product # A38556).



RICTOR Antibody (MA5-15006)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with RICTOR siRNA and decrease in signal intensity was observed in Western Blot application using Anti-RICTOR Polyclonal Antibody (Product # MA5-15006). {KD}



RICTOR Antibody (MA5-15006) in WB

Western Blot was performed using Anti-RICTOR Monoclonal Antibody (J.644.6) (Product # MA5-15006) and a 200 kDa band corresponding to Rapamycin-insensitive companion of mTOR was observed in A549 cells treated with MG132 (5  $\mu$ M for 4hr). Freshly prepared Whole cell extracts (30  $\mu$ g lysate) of A549 (Lane 1), A549 treated with MG132 (5  $\mu$ M for 4hr) (Lane 2) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The Blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).

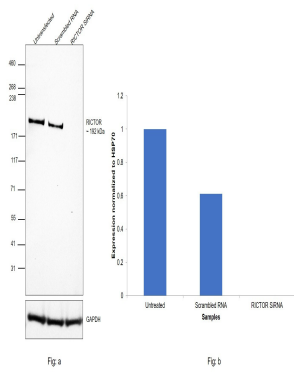
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**RICTOR Antibody (MA5-15006) in WB**

Knockdown of RICTOR was achieved by transfecting HeLa with RICTOR specific siRNAs (Silencer® select Product # s48408, S226001). Western blot analysis (Fig. a) was performed using Whole cell extracts from the RICTOR knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with RICTOR Polyclonal Antibody (Product # MA5-15006, 1:1000) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to RICTOR.



**RICTOR Antibody (MA5-15006)**

Altered expression of proteins upon cell treatment demonstrates antibody specificity. Western blot using Rictor Monoclonal Antibody (Product # MA5-15006), shows that HCT116 serum starved cells treated with EGF and upon treatment with Curcumin showed a decrease in expression for RICTOR as expected. In addition, there was an increase in the expression of RICTOR in A549 cell lines upon treatment with MG132. {TM}

