ROCKLAND
Immunochemicals for Research

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Certificate of Analysis

Product: Affinity Purified Anti-MAD2L1 [Rabbit]

Code: 600-401-461 **Lot #:** 16481

Size: 100 μg **Physical State:** Liquid (sterile filtered)

Antibody Concentration: 1.19 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None **Preservative:** 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

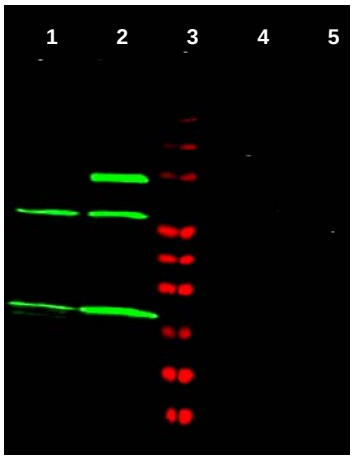

Figure. Western blot using Rockland's Affinity Purified anti-MAD2L1 antibody shows detection of a predominant band at ~24 kDa corresponding to MAD2L1 (arrowhead) present in Jurkat (lane 1) and HeLa (lane 2) whole cell lysates using the 800 nm channel (green). The identity of the higher molecular weight bands is unknown, although they may represent complexes of MAD2L1 with related binding proteins. Specific band reactivity is blocked when the antibody is pre-incubated with immunizing peptide (lanes 4 and 5 respectively) which completely blocks antibody staining. ~ 35 μg of lysate was separated on a 4-20% Tris-glycine gel by SDS-PAGE and transferred onto nitrocellulose. After blocking the membrane was probed with the primary antibody diluted to 1:1200. Incubation was 2h at room temperature followed by washes and reaction with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Rabbit IgG [H&L] MXHu (611-432-122) for 45 min at room temperature. Molecular weight markers were used for size comparison using the 700 nm channel (lane 3). IRDye™800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

Background: MAD2L1 (also called mitotic spindle assembly checkpoint protein, MAD2A, MAD2-like 1 and HsMAD2) is a component of the mitotic spindle assembly checkpoint monitors the process of kinetochore-spindle attachment and delays the onset of anaphase when this process is not complete. MAD2L1 inhibits the activity of the anaphase-promoting complex by sequestering CDC20 until all chromosomes are aligned at the metaphase plate. MAD2L1 is related to the MAD2L2 gene located on chromosome 1. A MAD2 pseudogene has been mapped to chromosome 14. This protein has a nuclear localization.

Application Note(s): This affinity purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a predominant band at ~ 24 kDa corresponding to full length protein by western blotting in the appropriate cell lysate or extract.

Recommended Dilutions:

ELISA	1:2,000 - 1:10,000
WESTERN BLOT	1:500 - 1:2,000
IF MICROSCOPY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity purified antibody is directed against human MAD2L1 protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with this protein from human, dog, macaque, chimpanzee and gecko based on 100% homology for the immunogen sequence. Cross reactivity with MAD2L1 may occur from mouse and chicken sources, as only a two amino acid residue change is found within the immunogen sequence (90% positive by BLAST). Cross reactivity with MAD2L1 homologues from other sources has not been determined.

Relevant Link(s): Swiss Prot: Q13257 NCBI Link NP_002349

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acid residues 3-13 of Human MAD2L1 protein.

Protein sequence: Human MAD2L1, 205 amino acids, predicated MW 23.5 kDa.

1	ma**lqlsreqg**	**itl**rgsaeiv	aeffsfgins	ilyqrgiyps	etftrvqkyg	ltllvttdle
61	likylnnvve	qlkdwlykcs	vqklvvvisn	iesgevlerw	qfdiecdkta	kddsapreks
121	qkaiqdeirs	virqitatvt	flpllevscs	fdlliytdkd	lvvpekwees	gpqfitnsee
181	vrlrsfttti	hkvnsmvayk	ipvnd			

General References:

Wang,R.H., Yu,H. and Deng,C.X. (2004) A requirement for breast-cancer-associated gene 1 (BRCA1) in the spindle checkpoint. *Proc. Natl. Acad. Sci. U.S.A.* **101** (49), 17108-17113.

Wu,C.W., Chi,C.W. and Huang,T.S. (2004) Elevated level of spindle checkprotein MAD2 correlates with cellular mitotic arrest, but not with aneuploidy and clinicopathological characteristics in gastric cancer. *World J. Gastroenterol*. **10** (22), 3240-3244.

Li,G.Q. and Zhang,H.F. (2004) Mad2 and p27 expression profiles in colorectal cancer and its clinical significance. *World J. Gastroenterol*. **10** (21), 3218-3220.

Sze,K.M., Ching,Y.P., Jin,D.Y. and Ng,I.O. (2004) Association of MAD2 expression with mitotic checkpoint competence in hepatoma cells. *J. Biomed. Sci*. **11** (6), 920-927.

Hernando,E., Nahle,Z., Juan,G., Diaz-Rodriguez,E., Alaminos,M., Hemann,M., Michel,L., Mittal,V., Gerald,W., Benezra,R., Lowe,S.W. and Cordon-Cardo,C. (2004) Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. *Nature* **430** (7001), 797-802.

Related Products:

#600-401-461	Affinity Purified Anti-Human MAD2L1 (Rabbit)
#600-401-470	Affinity Purified Anti-Human MAD2L2 (Rabbit)
#W09-000-364	HeLa Whole Cell Lysate
#W09-000-370	Jurkat Whole Cell Lysate
W09-001-367	HeLa Cell Nuclear Extract
#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	***Max*Tag**™ Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.