

# Product Datasheet

## RMND5A Antibody - BSA Free

### NBP1-92337

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP1-92337**

RMND5A Antibody - BSA Free

**Product Information**

<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	Concentrations vary lot to lot. See vial label for concentration. If unlisted please contact technical services.
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS (pH 7.2) and 40% Glycerol

**Product Description**

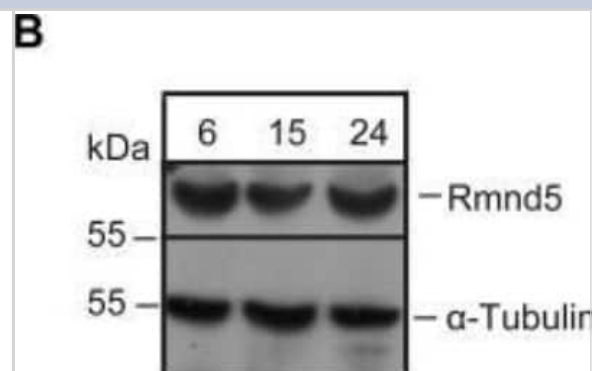
<b>Description</b>	Novus Biologicals Rabbit RMND5A Antibody - BSA Free (NBP1-92337) is a polyclonal antibody validated for use in IHC, WB and IP. Anti-RMND5A Antibody: Cited in 5 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Rabbit
<b>Gene ID</b>	64795
<b>Gene Symbol</b>	RMND5A
<b>Species</b>	Human, Mouse, Amphibian
<b>Reactivity Notes</b>	Frog reactivity reported in scientific literature (PMID: 25793641), Mouse reactivity reported in scientific literature (bioRxiv, DOI:10.1101/745174)
<b>Immunogen</b>	This antibody was developed against Recombinant Protein corresponding to amino acids: FDSDISSVGIDGCWQADSQRLLNEVMVEHFFRQGMLDVAE

**Product Application Details**

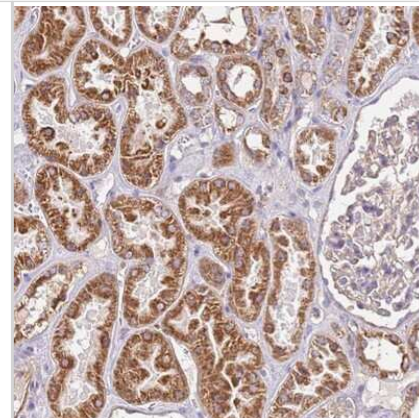
<b>Applications</b>	Western Blot, Immunohistochemistry-Paraffin, Immunohistochemistry
<b>Recommended Dilutions</b>	Western Blot 0.04 - 0.4 ug/ml, Immunohistochemistry 1:200 - 1:500, Immunohistochemistry-Paraffin 1:200 - 1:500
<b>Application Notes</b>	For IHC-Paraffin, HIER pH 6 retrieval is recommended.

**Images**

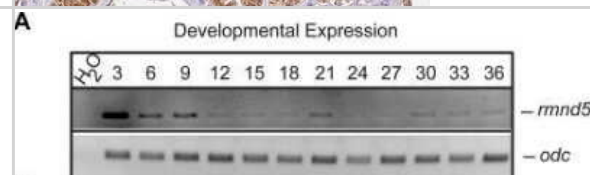
Western Blot: RMND5A Antibody [NBP1-92337] - rmnd5 is expressed during early embryonic development. Rmnd5 protein at different developmental stages. Western blot analysis of embryo lysate from indicated stages (top). alpha-RMND5A (Novus Biological; rabbit, 1:1000); alpha-Tubulin (at, 1:2500). Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0120342>), licensed under a CC-BY license.



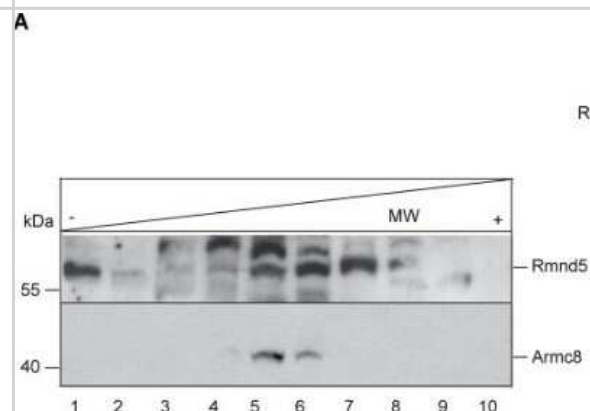
**Immunohistochemistry-Paraffin: RMND5A Antibody [NBP1-92337] -** Staining of human kidney shows strong cytoplasmic positivity in cells in tubules.



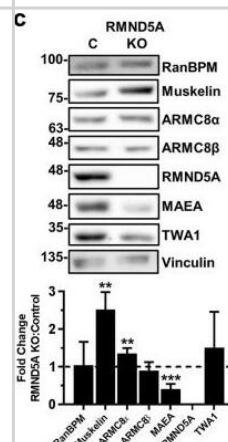
**Western Blot: RMND5A Antibody [NBP1-92337] -** *rmnd5* is expressed during early embryonic development. Temporal RT-PCR analysis of *rmnd5* expression (top panel); different developmental stages (NF-stages) indicated at the top. ODC1 functions as RNA input control (bottom). Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0120342>), licensed under a CC-BY license.



**Western Blot: RMND5A Antibody [NBP1-92337] -** *Rmnd5* is part of an ubiquitin ligase complex. Glycerol step gradient of *Xenopus laevis* NF stage 36 embryo lysates. Molecular mass (MW) standard: albumin (67 kDa), fraction 1, 2; LDH (140 kDa), fraction 4; catalase (232 kDa), fraction 6,7. Western blot analysis with alpha-RMND5A (*Rmnd5*; upper panel) (1:1000) and alpha-ARMC8 (lower panel) (1:1000). Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0120342>), licensed under a CC-BY license.



**RanBPM & TWA1 are essential for complex stability.** Whole cell extracts prepared from control shRNA & RanBPM shRNA HEK293 cells (a), or from control (labelled as C), TWA1, RMND5A, MAEA, ARMC8 & muskelin HEK293 CRISPR knockout cells (b–f) were analyzed by Western blot with antibodies to CTLH complex members, as indicated. Vinculin was used as a loading control. Quantifications are shown below each blot & protein levels are shown relative to control cells set to 1 & normalized to Vinculin levels. Data represent averages from three separate experiments, with error bars indicating SD. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31285494>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

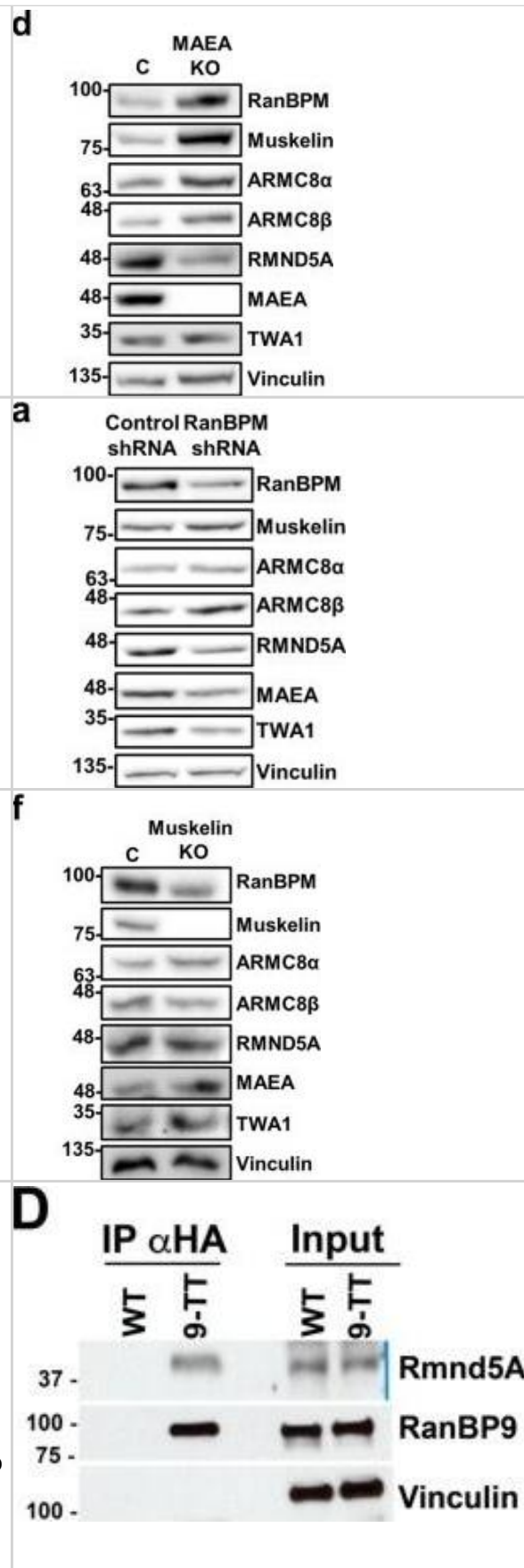


RanBPM & TWA1 are essential for complex stability. Whole cell extracts prepared from control shRNA & RanBPM shRNA HEK293 cells (a), or from control (labelled as C), TWA1, RMND5A, MAEA, ARMC8 & muskulin HEK293 CRISPR knockout cells (b–f) were analyzed by Western blot with antibodies to CTLH complex members, as indicated. Vinculin was used as a loading control. Quantifications are shown below each blot & protein levels are shown relative to control cells set to 1 & normalized to Vinculin levels. Data represent averages from three separate experiments, with error bars indicating SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31285494>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

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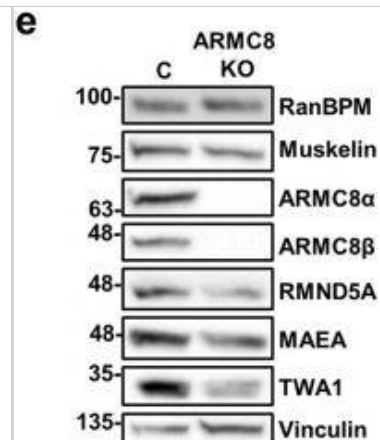
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Western Blot: RMND5A Antibody [NBP1-92337] - V5-HA-tagged RanBP9 maintains its ability to interact with known members of the CTLH complex & Nucleolin. RanBP9 WT & TT immortalized Mouse Embryonic Fibroblasts (MEFs) were cultured in standard conditions & protein lysates were obtained. Resin conjugated with  $\alpha$ HA antibodies was used to immunoprecipitate RanBP9-TT protein. IPed fractions & 5% of input were run on gels to generate 5 different membranes that were probed with the indicated antibodies by WB. Vinculin is used as loading control. Shown results are representative of two independent experiments (biological replicates). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32346083>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

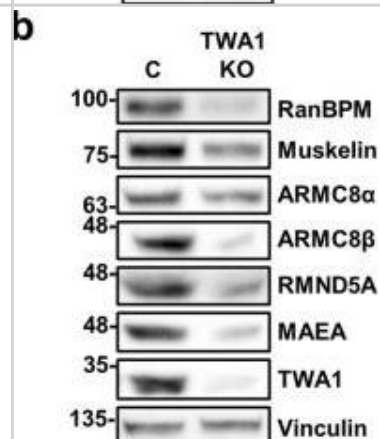




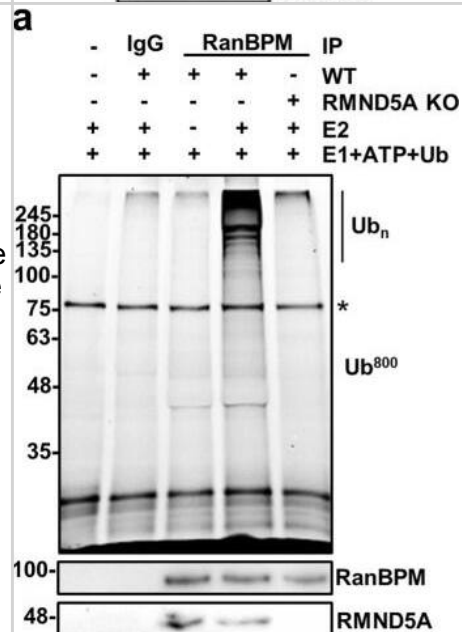
**Western Blot: RMND5A Antibody [NBP1-92337]** - RanBPM & TWA1 are essential for complex stability. Whole cell extracts prepared from control shRNA & RanBPM shRNA HEK293 cells (a), or from control (labelled as C), TWA1, RMND5A, MAEA, ARMC8 & muskelin HEK293 CRISPR knockout cells (b–f) were analyzed by Western blot with antibodies to CTLH complex members, as indicated. Vinculin was used as a loading control. Quantifications are shown below each blot & protein levels are shown relative to control cells set to 1 & normalized to Vinculin levels. Data represent averages from three separate experiments, with error bars indicating SD. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31285494>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



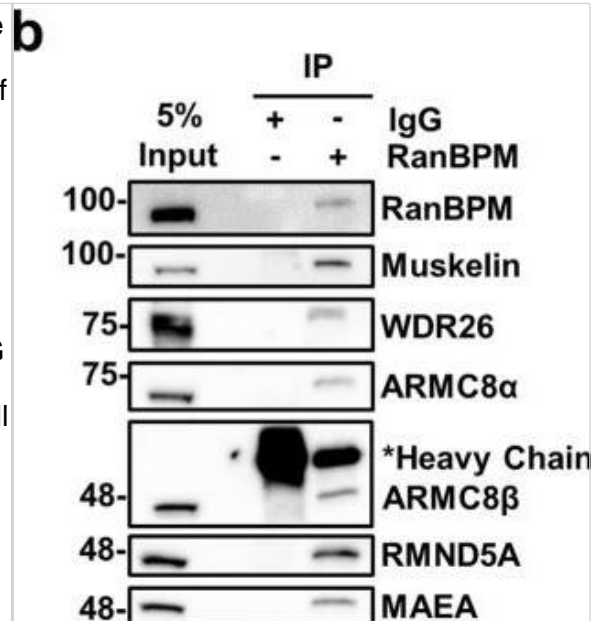
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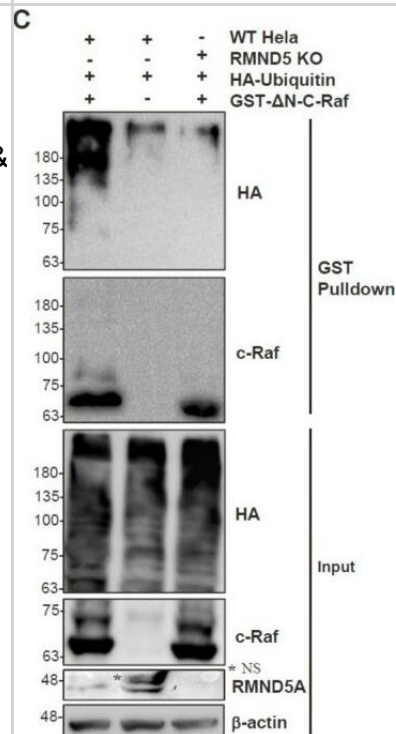
**Western Blot: RMND5A Antibody [NBP1-92337]** - The CTLH complex has E3 ligase activity. (a) RanBPM immunocomplexes have E3 ligase activity that is dependent on RMND5A. Whole cell extracts prepared from wild type (WT; lanes 3–4) or RMND5A KO (lane 5) HEK293 cells were subjected to immunoprecipitation with a RanBPM antibody or an IgG control, as indicated. Immunoprecipitates were resuspended in an ubiquitination assay master mix & E2 enzyme (UBE2D2) was added (+) or omitted (–), prior to incubation at 37 °C for 30 minutes. Reactions were run on SDS-PAGE gel & fluorescein-ubiquitin was imaged directly on the gel at 800 nm. Shown below is a Western blot analyzing RanBPM & RMND5A presence in the immunoprecipitates. Asterisk indicates a non-specific band. (b) MAEA is required for CTLH complex activity. RanBPM was immunoprecipitated from control or MAEA KO HEK293 cells & ubiquitination reactions were carried out as described above. The presence of RanBPM & MAEA in the immunoprecipitates was analyzed by Western blot. Asterisk indicates a non-specific band. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31285494>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: RMND5A Antibody [NBP1-92337] - Characterization of the CTLH complex.** (a) Schematic representation of the CTLH complex. The model is adapted from the yeast Gid complex<sup>26</sup>. Note that the position of muskelin in the complex has not been formally defined. (b) Subunits of the CTLH complex are present in RanBPM immunocomplexes. HEK293 whole cell extracts were incubated with a RanBPM antibody & immunoprecipitated. Immunoprecipitates were analyzed by Western blot with the indicated antibodies. IgG was used as a negative control. (c) WDR26 associates with the CTLH complex. Whole cell extracts were prepared from HeLa cells untransfected (-) or transfected with FLAG-tagged WDR26 (+). FLAG-WDR26 was immunoprecipitated with a FLAG antibody & immunoprecipitates were analyzed by Western blot with the indicated antibodies. (d) GID4 associates with CTLH complex. Whole cell extracts were prepared from HEK293 cells untransfected (-) or transfected with HA tagged GID4 (+). HA-GID4 was immunoprecipitated with an HA antibody & immunoprecipitates were analyzed by Western blot with the indicated antibodies. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31285494>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



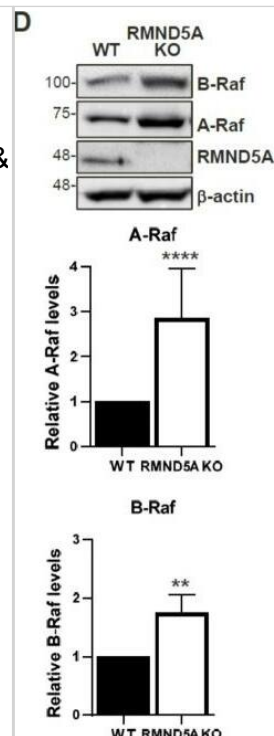
**Western Blot: RMND5A Antibody [NBP1-92337] - C-Raf is regulated by the proteasome through the CTLH complex.** Non-targeting shRNA control & shRNA RanBPM cells were treated with 10  $\mu$ M MG132 or DMSO, as vehicle, for 24 h. RIPA buffered whole cell extracts of HeLa (A), & HCT116 (B) were analyzed by Western blot with RanBPM, c-Raf &  $\beta$ -actin antibodies to detect RanBPM, c-Raf &  $\beta$ -actin proteins, respectively. c-Raf protein levels were normalized to  $\beta$ -actin levels. Quantifications of relative c-Raf protein levels are shown with error bars indicating SD (n = 4). \* p < 0.05; \*\* p < 0.01; ns, no significance. (C) c-Raf is ubiquitinated in a CTLH complex-dependent manner. HeLa control (WT HeLa) & RMND5A KO cells were transfected with GST- $\Delta$ N-c-Raf and/or HA-Ubiquitin as indicated. GST pull-downs performed using whole cell extracts were analyzed by Western blot with the indicated antibodies. Input represent 5% of the extracts used for pull-down. The asterisk (\*) indicates a non-specific band (NS). (D) A-Raf & B-Raf expression is increased in RMND5A KO cells. Extracts from HEK293 control & RMND5A CRISPR KO cells were analyzed by Western blot with the indicated antibodies. Quantifications are shown below with error bar indicating SD, n = 8 for A-Raf, \*\*\*\* p < 0.0001 & n = 3 for B-Raf, \*\* p < 0.01. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30795516>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



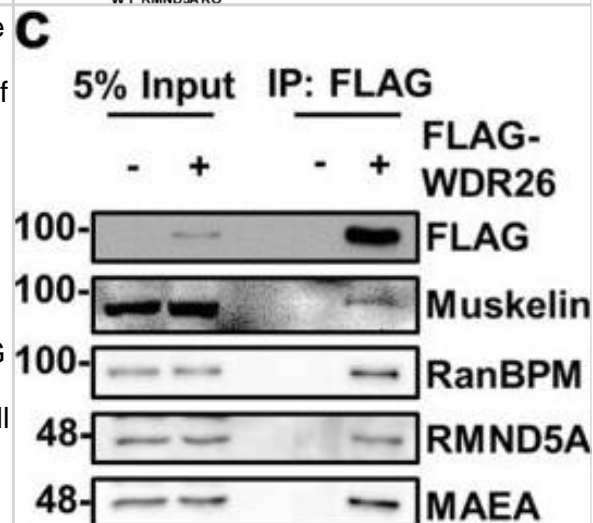




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## Publications

SHA Soliman, AE Stark, ML Gardner et al. Tagging enhances histochemical and biochemical detection of Ran Binding Protein 9 in vivo and reveals its interaction with Nucleolin Sci Rep, 2020;10(1):7138. 2020-01-01 [PMID: 32346083] (WB, IP, Mouse)

Soliman S, Stark AE, Gardner M, Harshman SW Tagging allows faithful tracing of expression and enhances biochemical detection of Ran Binding Protein 9 in vivo bioRxiv (IP, Mouse)

Maitland MER, Onea G, Chiasson CA et al. The mammalian CTLH complex is an E3 ubiquitin ligase that targets its subunit muskellin for degradation Sci Rep 2019-07-08 [PMID: 31285494] (WB, Human)

McTavish CJ, Berube-Janzen W, Wang X et al. Regulation of c-Raf Stability through the CTLH Complex Int J Mol Sci 2019-02-21 [PMID: 30795516] (WB, Human)

Salemi LM, Maitland MER, Yefet ER, Schild-Poulter C. Inhibition of HDAC6 activity through interaction with RanBPM and its associated CTLH complex BMC Cancer 2017-07-01 [PMID: 28668087] (WB, Human)

Pfaffmann T, Villavicencio-Lorini P, Subudhi AK et al. RMND5 from *Xenopus laevis* Is an E3 Ubiquitin-Ligase and Functions in Early Embryonic Forebrain Development. PLoS ONE. 2015-03-21 [PMID: 25793641] (WB, Frog)





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Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP1-92337**

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NBP1-92337PEP	RMND5A Recombinant Protein Antigen
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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