Product Datasheet

AIP/ARA9 Antibody (35-2)
NB100-127

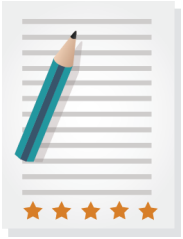
Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

Reviews: 4 Publications: 32

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-127

Updated 4/13/2025 v.20.1



NB100-127

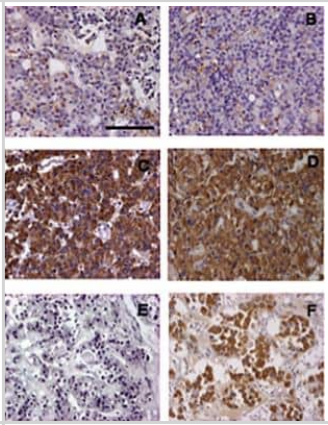
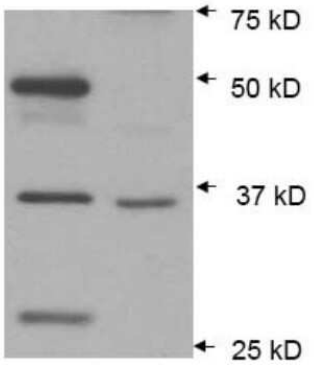
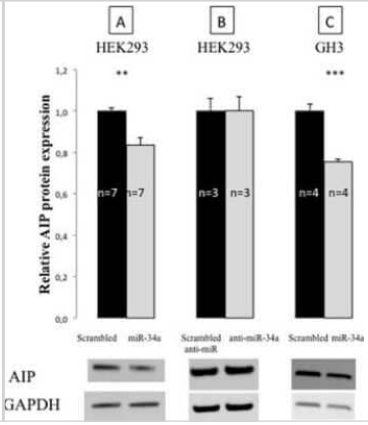
AIP/ARA9 Antibody (35-2)

Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	35-2
Preservative	0.1% Sodium Azide
Isotype	IgG1
Purity	Tissue culture supernatant
Buffer	Tissue culture supernatant
Target Molecular Weight	37 kDa

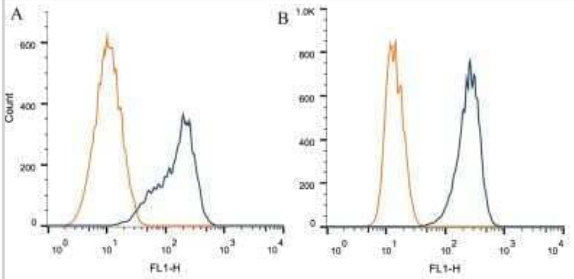
Product Description	
Host	Mouse
Gene ID	9049
Gene Symbol	AIP
Species	Human, Mouse, Rat, Primate
Reactivity Notes	Customers have reported success on rat lysate (see review). Primate reactivity reported in scientific literature (PMID: 10986286).
Immunogen	Bacterially expressed human AIP/ARA9 [UniProt# O00170]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Chromatin Immunoprecipitation Sequencing
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:200, Flow Cytometry 1 ug per million cells, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 23702468), Immunoprecipitation 1:10-1:100, Immunohistochemistry-Paraffin 1:500 - 1:1000, Chromatin Immunoprecipitation (ChIP) 1:10-1:500, Chromatin Immunoprecipitation Sequencing reported in scientific literature (PMID 21984905)
Application Notes	In WB, a band is seen at 37 kDa, representing AIP/ARA9. It is specific for the FKBP domain. For IHC-P, this product has been used at 1:500 - 1:1000 dilution range (PMIDs: 22659247, 25019383, 23940012) . In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Hek293 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:200, apparent MW was 44 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

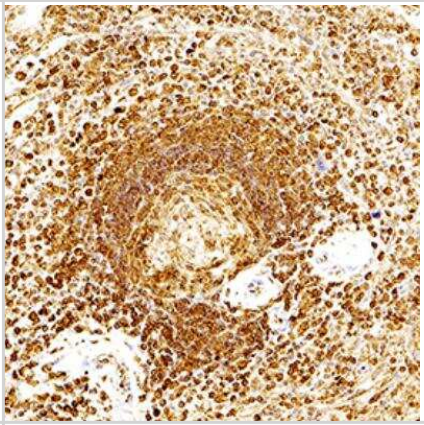
Images

Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Fig 1. Aryl hydrocarbon receptor interacting protein (AIP) immunostaining. A and B Examples of low AIP expression; C and D: Examples of high AIP expression; E Normal human pituitary staining with omitting primary antibody (negative control); F Normal human pituitary staining with AIP (positive control); Scale bar = 1000 um. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107) licensed under a CC-BY license.	
Simple Western: AIP/ARA9 Antibody (35-2) [NB100-127] - Simple Western lane view shows a specific band for AIP/ARA9 in 0.5 mg/ml of Hek293 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: AIP/ARA9 Antibody (35-2) [NB100-127] - Detection on HEK 293T/17 cells. Lane 1: IP ARA9, WB ARA9, Lane 2: WB ARA9 with whole cell lysate. Photo courtesy of Liling Zeng, Boston University.	
Western Blot: AIP/ARA9 Antibody (35-2) [NB100-127] - Effect of miR-34a (A) and anti-miR-34a (B) on endogenous AIP protein levels in HEK293 and in GH3 (C) cells 48 hours after transfection. Data are shown as mean +/- SEM, **, P<0.01 ***, P<0.001. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/25658813/) licensed under a CC-BY license.	

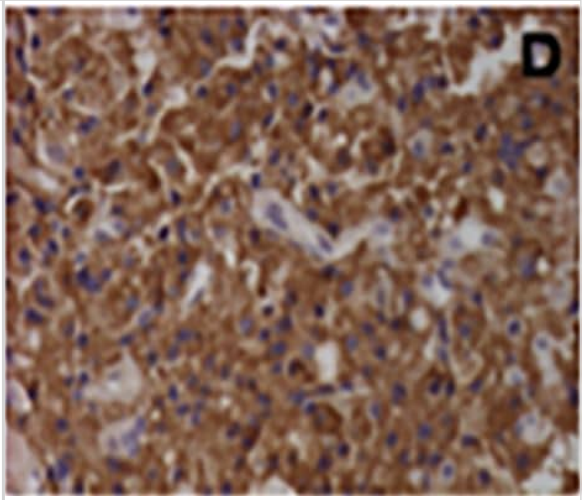
Flow Cytometry: AIP/ARA9 Antibody (35-2) [NB100-127] - Intracellular flow cytometric staining of 1 x 10^6 CHO (A) and MCF-7 (B) cells using AIP/ARA9 antibody (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10^6 cells was used.



Immunohistochemistry-Paraffin: AIP/ARA9 Antibody (35-2) [NB100-127] - AIP/ARA9 was detected in immersion fixed paraffin-embedded sections of human spleen using Mouse Anti-Human AIP/ARA9 (35-2) Monoclonal Antibody (Catalog # NB100-127) at 1:300 for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm in splenocytes.

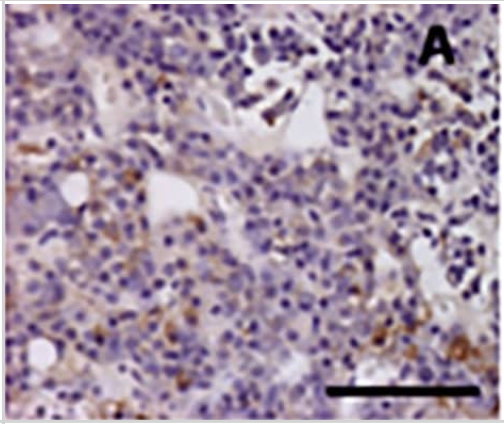


EPS15 is targeted via a SPOP/SPOPL binding consensus motif.(A) Cartoon of human EPS15 domain-organization and the amino-acid sequence. Indicated by color code are the SPOP/SPOPL binding site (red) and the lysine residue (yellow), which is ubiquitinated in a CRL3SPOPL–dependent manner in vivo. In addition, the amino-terminal Ca2+-binding EF-hand motifs (EH), the coiled-coil domain involved in dimerization and the two carboxy-terminal ubiquitin-interacting motifs (UIMs) involved in ubiquitin-binding are indicated. (B) EPS15 ubiquitin-profiling. Peptides containing EPS15 modification sites were quantified with LC-MS/MS after enrichment of the K-ε-GG motif from whole cell digests of HeLa cells treated with siSPOPL or siControl. Normalized precursor mass intensity profiles for EPS15 sites corresponding to K793, K801 and K693 are shown (raw data in Figure 4—figure supplement 1B). Quantification of the β-Actin K113 and the polyubiquitin K11 linkage peptide control for comparable enrichment. Data are mean ± SD, N = 3. **p≤0.01. (C) Purified SPOPL was incubated as indicated with GST-tagged wild-type EPS15 or GST-EPS15 mutants, where the predicted SPOPL binding motifs have been mutated individually (GST-EPS15S605-S607A and EPS15S744-S746A, respectively), pulled down with glutathione sepharose (IP [GST]) and bound proteins were analyzed by Coomassie blue staining (upper panel) and immunoblotting (lower panels). Note that SPOPL readily binds to GST-EPS15 and GST-EPS15S605-S607A, but this interaction is strongly reduced with the GST-EPS15S744-S746A mutant. (D) HeLa cells stably expressing GFP-tagged wild-type EPS15, the EPS15S744-S746A or the EPS15K793R mutants from a doxycycline-inducible promoter were transfected as indicated (+) with control siRNA or siRNA depleting SPOPL. The levels of EPS15-GFP, EGFR and for control tubulin (TUB) were analyzed by immunoblotting with specific antibodies. Experiments were quantified in Fiji and the EPS15 levels plotted as fold-increase compared to controls. Data are mean ± SEM, N = 4. *p≤0.05. Note that SPOPL depletion does not further increase the levels of both EPS15 mutants. (E) Total cell extracts were prepared from HeLa cells expressing either GFP-tagged

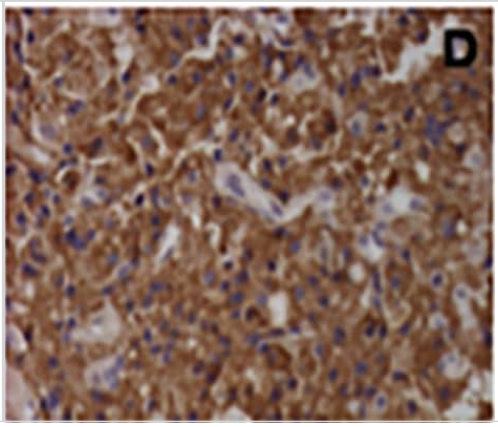


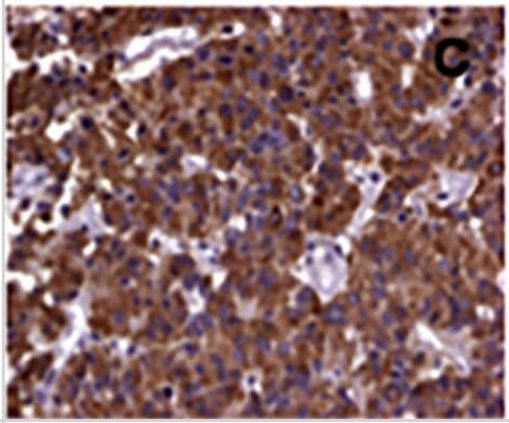
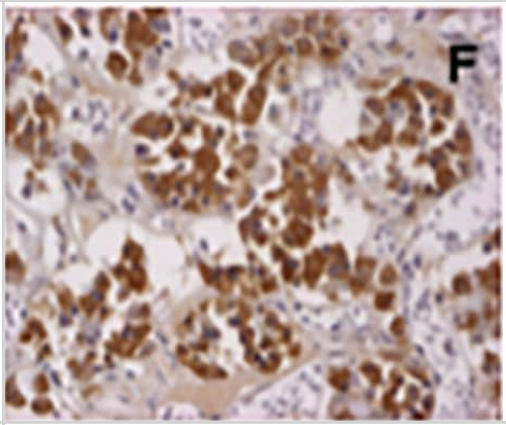
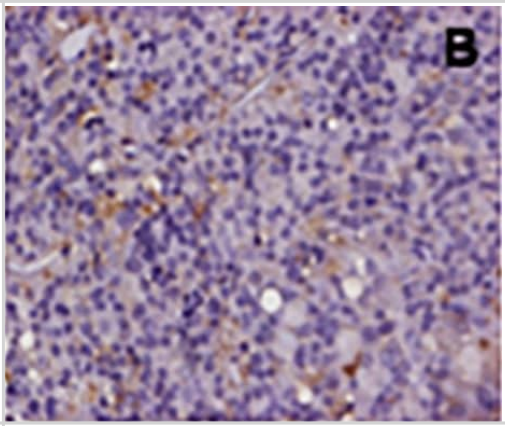
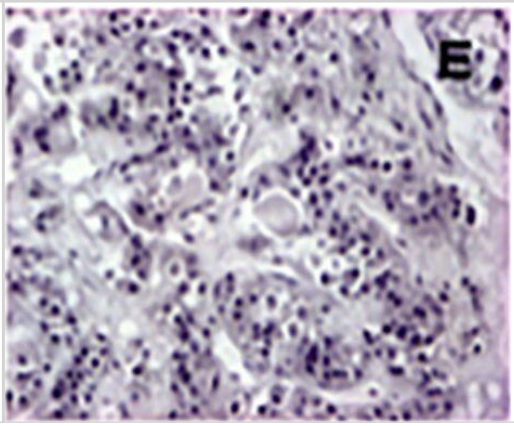
wild-type, the EPS15S744-S746A mutant or the EPS15K793R mutant in the presence (+) or absence (-) of HA-tagged SPOPL overexpression. The levels of EPS15-GFP, SPOPL-HA and control GAPDH were analyzed by immunoblotting. Note that overexpression of SPOPL-HA is able to induce degradation of wild-type but not the EPS15S744-S746A-GFP or the EPS15K793R-GFP mutant.DOI:https://dx.doi.org/10.7554/eLife.13841.009EPS15 is targeted via a SPOP/SPOPL binding consensus motif.(A) Alignments of the carboxy-terminal domains of EPS15 proteins from various species. Conserved SPOPL-binding motifs and putative ubiquitination sites are highlighted by yellow boxes. (B) Peptides containing EPS15 modification sites were quantified with LC-MS/MS after enrichment of the K-ε-GG motif from whole cell HeLa digests treated with siSPOPL and siControl. Raw intensities for each of the triplicate LC-MS/MS runs are shown with each of the siControl conditions scaled to 100% intensity. Normalized precursor mass intensity profiles for EPS15 sites corresponding to K793, K801 and K693 are shown, with only K793 showing significant downregulation in the depletion condition. Quantification of a peptide corresponding to β-Actin K113 and the poly-ubiquitin K11 linkage peptide is also shown to demonstrate that enrichment variations did not influence the quantification of the EPS15 sites. Additionally, the total ion chromatographic intensities for each run are plotted to provide insight into the consistency of each of the separate experiments performed on different days. Data are mean ± SD, N = 3. (C) HeLa cell lines stably expressing wild-type EPS15-GFP, the EPS15S744-746A-GFP mutant or the EPS15K793R-GFP mutant from the inducible doxycycline-promoter were treated with doxycycline for 3 days, and analyzed by live cell imaging. Displayed are maximal projections of Z-stack acquisitions, fully covering cell height. Scale bar = 10 μm.DOI:https://dx.doi.org/10.7554/eLife.13841.010 Image collected and cropped by CiteAb from the following open publication (https://elifesciences.org/articles/13841), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 μm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 μm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunohistochemistry: AIP/ARA9 Antibody (35-2) [NB100-127] - Aryl hydrocarbon receptor interacting protein (AIP) immunostaining.A & B—Examples of low AIP expression; C & D: Examples of high AIP expression; E—Normal human pituitary staining with omitting primary antibody (negative control); F—Normal human pituitary staining with AIP (positive control); Scale bar = 1000 µm. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0117107), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Fukuda T, Tanaka T, Hamaguchi Y et Al. Augmented Growth Hormone Secretion and Stat3 Phosphorylation in an Aryl Hydrocarbon Receptor Interacting Protein (AIP)-Disrupted Somatotroph Cell Line PLoS One 2016-10-05 [PMID: 27706259] (Immunohistochemistry, Western Blot)

De Sousa, S M C, McCabe, M J Et al. Germline variants in familial pituitary tumour syndrome genes are common in young patients and families with additional endocrine tumours. Eur J Endocrinol 2017-05-01 [PMID: 28220018] (IP, Human)

HernAndez-RamIrez L C, Morgan R M L et al. Multi-chaperone function modulation and association with cytoskeletal proteins are key features of the function of AIP in the pituitary gland. Oncotarget 2018-06-02 [PMID: 29507682] (ICC/IF, Human)

Kasuki L, Wildemberg L E et al. MANAGEMENT OF ENDOCRINE DISEASE: Personalized medicine in the treatment of acromegaly. Eur J Endocrinol 2018-01-03 [PMID: 29339530] (IF/IHC, Human)

Lee HJ, Jung YH, Choi GE et al. Urolithin A suppresses high glucose-induced neuronal amyloidogenesis by modulating TGM2-dependent ER-mitochondria contacts and calcium homeostasis Cell Death Differ. 2020-07-23 [PMID: 32704090] (Human)

Hage C, Sabini E, Alsharhan H, et al. Acromegaly in the setting of Tatton-Brown-Rahman Syndrome Pituitary 2019-12-19 [PMID: 31858400]

Sun D, Stopka-Farooqui U, Barry S, Aksoy E et al. Aryl Hydrocarbon Receptor Interacting Protein Maintains Germinal Center B Cells through Suppression of BCL6 Degradation Cell Rep 2019-04-30 [PMID: 31042473] (WB, Mouse)

Yamamoto R, Robert Shima K, Igawa H et al. Impact of preoperative pasireotide therapy on invasive octreotide-resistant acromegaly Endocr. J. 2018-08-04 [PMID: 30078825] (IF/IHC, Human)

Ozkaya HM, Comunoglu N, Sayitoglu M, Keskin FE. Germline mutations of aryl hydrocarbon receptor-interacting protein (AIP) gene and somatostatin receptor 1-5 and AIP immunostaining in patients with sporadic acromegaly with poor versus good response to somatostatin analogues. Pituitary. 2018-02-17 [PMID: 29455389] (Human)

Ritvonen E, Pitkanen E, Karppinen A et al. Impact of AIP and inhibitory G protein alpha 2 proteins on clinical features of sporadic GH-secreting pituitary adenomas. Eur. J. Endocrinol. 2017-02-01 [PMID: 27998919] (IF/IHC, Human)

Rotondi S, Modarelli A, Oliva MA et al. Expression of Peroxisome Proliferator-Activated Receptor alpha (PPARalpha) in somatotropinomas: Relationship with Aryl hydrocarbon receptor Interacting Protein (AIP) and in vitro effects of fenofibrate in GH3 cells. Mol. Cell. Endocrinol. 2016-02-10 [PMID: 26872613] (IHC-P, Human)

Iacovazzo D, Carlsen E, Lugli F et al. Factors predicting pasireotide responsiveness in somatotroph pituitary adenomas resistant to first generation somatostatin analogues: an immunohistochemical study. Eur. J. Endocrinol. 2015-11-19 [PMID: 26586796] (IHC-P, Human)

More publications at http://www.novusbio.com/NB100-127

Procedures

Protocol specific for ARA9 (35-2) Antibody (NB100-127)

1. Wash plate twice with cold PBS.
2. Add 1 mL lysis buffer into P100 plate, sit on ice for 20 min with gentle shaking, centrifuge 14,000rpm/10min/4C, take supernatant.
3. Preclear the lysate with 50 uL protein G slurry, tumble, 45 min/4C, followed by a centrifuge 14,000rpm/15min/4C.
4. Add 1 ug antibody (3 uL anti-ARA9 antibody I used) to 50 uL protein G slurry, add 500 uL cold PBS, tumble, 1 hr/4C. Wash Ab/beads twice by adding PBS. Spin down beads by centrifuge at 1000g/1 min.
5. Add precleared lysate into pre-bond Ab/protein G complex, tumble O/N, 4C Spin down beads by centrifuge at 1000g/1 min.
6. Wash beads five times with lysis buffer.
7. Add SDS sample buffer to beads.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NB100-127

NBL1-07415	AIP/ARA9 Overexpression Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-127

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications