Product Datasheet

Clostridium Difficile Toxin A Antibody (PCG4.1) - BSA Free NB600-1066-0.1mg

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.



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NB600-1066-0.1mg

Clostridium Difficile Toxin A Antibody (PCG4.1) - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.16 mg/ml
Storage	Store at 4C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	PCG4.1
Preservative	0.05% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein A purified
Buffer	10mM Sodium Phosphate (pH 7.4) and 0.15M NaCl
Product Description	
Host	Mouse
Species	Bacteria
Specificity/Sensitivity	C. difficile Toxin A. Does not cross react with C. difficile Toxin B.
Immunogen	Full length protein A (C. difficile).
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 1:100-1:2000, ELISA 1:100-1:2000, Immunocytochemistry/ Immunofluorescence
Application Notes	WB reactivity reported in (PMID: 28346491). Works with stool samples.

Images

Immunocytochemistry/Immunofluorescence: Clostridium Difficile Toxin A Antibody (PCG4.1) [NB600-1066] - TcdA colocalizes with PACSIN2 during entry in Caco-2 cells. Immunofluorescence assays were performed as described in Fig 1B. At indicated time points, cells were fixed, stained for PACSIN2 and analyzed by confocal microscopy. Merged images show PACSIN2 in red, toxin in green and colocalization in yellow. The images shown are representative of multiple fields imaged from three independent experiments. Image collected and cropped by CiteAb from the following publication

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Immunocytochemistry/Immunofluorescence: Clostridium Difficile Toxin A Antibody (PCG4.1) [NB600-1066] - TcdA and TcdB utilize distinct endocytic mechanisms to intoxicate colonic epithelial cells. TcdA does not colocalize with clathrin heavy chain during cell entry. Caco-2 cells on glass coverslips were chilled at 10C for 45 min and then exposed to media containing 50 nM TcdA-546 or buffer (no toxin control). The toxin was allowed to bind to cells for 45 min at 10C. Unbound toxin was removed, and cells were shifted to 37C to allow internalization of toxin for the times shown. At each time point, cells were washed once with prewarmed PBS, fixed and stained for CHC, and imaged by confocal microscopy. Merged images show clathrin in red and toxin in green. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.ppat.1006070), licensed under a CC-BY license.

Immunocytochemistry/Immunofluorescence: Clostridium Difficile Toxin A Antibody (PCG4.1) [NB600-1066] - Depletion of PACSIN2 inhibits TcdA entry in Caco-2 cells. Caco-2 cells expressing non-targeting shRNA (Ctrl shRNA) or shRNA 982 targeting PACSIN2 were incubated with 50 nM TcdA-546 at 10C for 45 min. Unbound toxins were removed and cells were shifted to 37C to allow internalization. After 20 min, cells were washed, fixed, stained for PACSIN2 and imaged by confocal microscopy. PACSIN2 and TcdA staining from ctrl shRNA and sh982 expressing cells are shown. The images shown are representative of multiple fields imaged from three independent experiments. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.ppat.1006070), licensed under a CC-BY license.

SPOP inhibits Hh/Gli signaling activity. (A) SPOP interacts with Gli2 protein in MKN28 cells by co-immunoprecipitation method. Goat antirabbit IgG is used as negative control. (B) Quantification of Gli1 and Gli2 mRNA in Myc-SPOP transfected MKN28 cells. (C) Increasing SPOP reduced Gli2 expression. AGS cells were transfected with different amount of Myc-SPOP for 48 h. Full length of Gli2 is detected in cell lysates by Western blotting. (D) SPOP promotes Gli2 degradation through proteasome pathway. MKN45 cells were treated with vehicle (DMSO) or miR-SPOP for 48 h, with or without 10 mM MG-132. In order to limit toxicity, MG-132 was added 4 h before cell harvest. Lysates were subjected to immunoblotting with indicated antibodies. (E) Dual luciferase reporter assay is performed in HEK293T cells. Myc-SPOP were transfected into the cells and lysed after 48 h incubation. The percentage of decrease in luciferase activity was calculated. (F) SPOP affects Gli2 abundance in cytoplasm. Immunofluorescent stainings of transfected Myc-SPOP and endogenous Gli2 were performed in MKN45 cells. MKN45 cells were transfected with Myc-SPOP for 48 h. Myc-SPOP was detected by incubating cells with mouse anti-Myc antibody and subsequently Alexa Fluor 594 goat anti-mouse antibody. Gli2 was detected by incubating cells with rabbit anti-Gli2 antibody and subsequently Alexa Fluor 488 donkey anti-rabbit antibody. Nucleus was identified by DAPI staining. Image collected and cropped by CiteAb from the following open publication

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Parkin protein levels are unaffected in liver while a small decrease is measured in G93A cerebellumRepresentative Western blot for Parkin and p62 in cerebellum (Cb) homogenates at 130 days. Quantification indicates that Parkin is decreased in G93A mice relative to Non Tg at 130 days. Protein levels were normalized by $\beta \Box$ actin. Results are expressed as mean \pm SEM and percent of Non Tq; n = 8 (four males and four females) mice per group. *P = 0.039 by paired Wilcoxon's test. Quantification of p62 in the homogenates indicates that p62 is increased in PKO and PKO/G93A mice, independent of SOD1 G93A expression, at 130 days of age. $\beta \Box$ actin was used for normalization. Results are expressed as mean ± SEM and percent of Non Tg; n = 8 (four males and four females) mice per group. No statistically significant differences were found between Non Tg and G93A by paired Friedman's test with Dunn's correction (P = 0.99). ***P = 0.0001 by paired Student's t Lest (G93A vs. PKO/G93A).Representative Western blot of Parkin and p62 in liver homogenates at 130 days. Parkin protein levels were guantified at 130 days. β actin was used for normalization. Results are expressed as mean \pm SEM and percent of Non Tg; n = 8 (four males and four females) mice per group. No statistically significant differences were found between Non Tg and G93A (P = 0.546 by paired Wilcoxon's test).Quantification of p62 protein levels in liver at 130 days. Results are expressed as mean \pm SEM and percent of Non Tg; n = 8 (four males and four females) mice per group. No statistically significant differences were found between Non Tg and G93A (P = 0.546 by paired Wilcoxon's test). No statistically significant differences were found among the other groups. Source data are available online for this figure. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/30126943), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Representative immunohistochemical expression for HIF-1a, c-Met, CA9 and GLUT1. HIF-1a is stained in cytoplasm shown with no staining in normal cervix (A), weak staining intensity in high grade CIN (B), and strong staining intensity in squamous cell carcinoma (C). c-Met (D-F), CA9 (G, H) and GLUT1 (I) shows cell membranous staining. Representative c-Met expression in cervical samples shown with no staining in normal cervix (D), weak membranous staining intensity in squamous cell carcinoma (E) and strong intensity in squamous cell carcinoma (F). CA9 expression showing moderate intensity staining in carcinoma in situ (CIS) (G) and strong staining in adenocarcinoma (H). GLUT1 expression showing strong intensity in squamous cell carcinoma (I). Scale bar: 50 µm. Image collected and cropped by CiteAb from the following open publication (https://translationalmedicine.biomedcentral.com/articles/10.1186/1479-5876-11-185), licensed under a CC-BY license. Not internally tested by Novus

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Dectin-1-induced TSLP negatively regulates pro-IL-1 β and HIF-1 α . (A) Human mDC were stimulated with SC glucan, CA glucan or heat killed C. albicans hyphae with anti-TSLPR antibodies or IgG isotype control for 24 h (n = 6 independent donors, presented as pooled data). Lactate production was measured in cell-culture supernatants using colourmetric L-lactate detection kit. (B) Human mDC were stimulated SC glucan with either anti-TSLP, anti-TSLPR or IgG isotype control antibodies for 8 h (n = 1 representative donor presented, three separate experiments performed). Pro-IL-1β, IL-1β, HIF-1α, phospho-p38 MAPK, p38 MAPK, phospho-AMPK, AMPK and β -actin were measured by immunoblot. (C–G) Densitometry of cumulative data was performed using Image Studio Lite software with pro-IL-1 β , IL-1 β and HIF-1 α normalized to β-actin and phospho-p38 MAPK and phospho-AMPK normalized to total p38 MAPK and AMPK respectively. Data is reported as percentage of maximal signal observed within each donor (n = 3 independent donors, presented as pooled data). Cumulative data displayed as mean +SEM. Statistical analysis calculated using one-way ANOVA with Bonferroni post-tests (***p = 0.001). Image collected and cropped by CiteAb from the following open publication

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Localization of candidate RNAs at telomeres. Localization of SNORD17 (A) and NEAT1 (B) at telomeres in human U-2 OS cells. Cells were fixed and sequentially incubated with Abs against TRF-2 and AlexaFluor 488conjugated anti-mouse IgG. Subsequently, the cells were hybridized with the RNA probes and subjected to fluorescence microscopy. Three different cells are shown. Image collected and cropped by CiteAb from the following open publication

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Ctrl shRNA

B





Publications

Ahmed UKB, Shadid TM, Larabee JL, Ballard JD. Combined and Distinct Roles of Agr Proteins in Clostridioides difficile 630 Sporulation, Motility, and Toxin Production mBio 2021-01-14 [PMID: 33443122] (WB)

Wang S, Heuler J, Wickramage I, Sun X Genomic and Phenotypic Characterization of the Nontoxigenic Clostridioides difficile Strain CCUG37785 and Demonstration of Its Therapeutic Potential for the Prevention of C. difficile Infection Microbiology spectrum 2022-03-22 [PMID: 35315695] (ELISA, Bacteria)

Details:

Clostridioides difficile

Zhu D, Wang S, Sun X. FliW and CsrA Govern Flagellin (FliC) Synthesis and Play Pleiotropic Roles in Virulence and Physiology of Clostridioides difficile R20291 Frontiers in microbiology 2021-10-05 [PMID: 34675903]

Ahmed UKB The Accessory Gene Regulator (Agr) System and Regulatory Network in Clostridioides Difficile Thesis 1905-07-13

Zhu D, Patabendige HMLW, Tomlinson BR et al. Cwl0971, a novel peptidoglycan hydrolase, plays pleiotropic roles in Clostridioides difficile R20291 Environmental microbiology 2021-04-24 [PMID: 33893759]

Oliveira PH, Ribis JW, Garrett EM et al. Epigenomic characterization of Clostridioides difficile finds a conserved DNA methyltransferase that mediates sporulation and pathogenesis Nat Microbiol 2019-11-25 [PMID: 31768029] (WB, Bacteria)

Chandrasekaran R, Kenworthy AK, Lacy DB et al. Clostridium difficile Toxin A Undergoes Clathrin-Independent, PACSIN2-Dependent Endocytosis PLoS Pathog. 2016-12-11 [PMID: 27942025] (WB, Human)

Edwards AN, Krall EG, McBride SM RstA Regulation of Clostridioides difficile Toxin Production and Sporulation in Phenotypically Diverse Strains bioRxiv 2019-09-18 [PMID: 31659010] (WB, C. difficile)

Woods E, Edwards A, McBride S. The C. difficile clnRAB operon initiates adaptations to the host environment in response to LL-37 PLoS Pathog. [PMID: 30125334] (WB, Bacteria)

Anjuwon-Foster BR, Maldonado-Vazquez N, Tamayo R. Et al. Characterization of Flagellum and Toxin Phase Variation in Clostridioides difficile Ribotype 012 Isolates J Bacteriol 2018-05-09 [PMID: 29735765] (WB, Bacteria)

Details:

Citation using the DyLight 800 version of this antibody.

Edwards A, Anjuwon-Foster B, McBride S. RstA is a Major Regulator of Clostridioides difficile Toxin Production and Motility. bioRxiv 2018-09-13 [PMID: 30862746] (WB, Bacteria)

Daou N, Wang Y, Levdikov VM et al. Impact of CodY protein on metabolism, sporulation and virulence in Clostridioides difficile ribotype 027 PLoS ONE 2019-01-30 [PMID: 30699117] (ELISA, Bacteria)

More publications at <u>http://www.novusbio.com/NB600-1066</u>

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

Products Related to NB600-1066-0.1mg

8619-GT-020	Clostridium Difficile Toxin A [Unconjugated]
NBP1-96981-0.5mg	Mouse IgG2a Kappa Isotype Control (M2AK)
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]

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