Datasheet for 200-401-C14

Flagellin Antibody

Overview

Description:	Anti-Flagellin (RABBIT) Antibody - 200-401-C14
Item No.:	200-401-C14
Size:	100 µg
Applications:	ELISA, WB, IF
Reactivity:	Borrelia burgdorferi B31
Host Species:	Rabbit

Product Details

Background:	Flagellin is a protein found in the hollow cylinder forming the filament in bacterial flagellum. Its structure is helical, which is important for its function. Studies comparing aflagellate Borrelia to flagellated indicate that the flagella have a role in the invasion of human tissue. The N- and C-termini of flagellin form the inner core of the flagellar filament, and the central portion of the protein makes up the outer surface. While the terminus of the protein is quite similar between all bacterial flagellins, the central portion is variable. The flagellin genes are highly conserved among the different Borrelia species. Mammals often have acquired immune responses (T-cell and antibody responses) to flagellated bacterium. Some bacteria are able to switch between multiple flagellin genes in order to evade this response. Borrelia burgdorferi, the spirochete that is associated with Lyme Disease, may use this tactic when challenging mammals with infection. Borrelia have double-stranded linear plasmids in addition to supercoiled circular plasmids, in low copy number. This suggests that initiation of DNA replication and partitioning are carefully controlled during the cell division cycle. It is believed that expression of the various proteins associated with the spirochete may be regulated by the changes in tick life cycle, changes in conditions during tick feeding (such as temperature, pH, and nutrients) and/or in coordination with the course of infection of the mammal host, i.e., changes in environment as the spirochete migrates from the tick's midgut to its salivary glands to the mammal host. B. burgdorferi can attach to (and also differentially express antigens in) diverse tissues within the vertebrate host and the tick vector, suggesting that physiological factors other than pH and temperature may play roles in modulating B. burgdorferi gene expression.
Synonyms:	rabbit anti-Flagellin Antibody, 41 kDa antigen, Borrelia burgdorferi p41, fla, Flagellar filament 41 kDa core protein, Bacterial flagellin.
Host Species:	Rabbit
Clonality:	Polyclonal

Format:	IgG

Target Details

Gene Name:	BBU94A_0149, fla
Reactivity:	Borrelia burgdorferi B31
Immunogen Type:	Recombinant Protein
Immunogen:	MBP-fusion protein corresponding to Borrelia burgdorferi Flagellin protein.
Purity/Specificity:	This product was Protein-A purified and cross-adsorbed against MBP from monospecific antiserum by chromatography. This antibody is specific for Lyme Borrelia spp. Flagellin protein. A BLAST analysis was used to suggest cross-reactivity with Flagellin from B. burgdorferi, garinii, and valaisiana sources based on 100% homology with the immunizing sequence.. Cross-reactivity with Flagellin from other sources has not been determined.
Relevant Links:	• UniProtKB - P11089 • NCBI - WP_002661938.1 • GeneID - 7106737

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF (Based on references)
Application Note:	This protein-A purified antibody has been tested for use in ELISA and Western blotting. Specific conditions for reactivity should be optimized by the user. Expect a band approximately 33.9 kDa in size corresponding to Borrelia burgdorferi Flagellin protein by Western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:6,000
WB:	1:1,000

Formulation

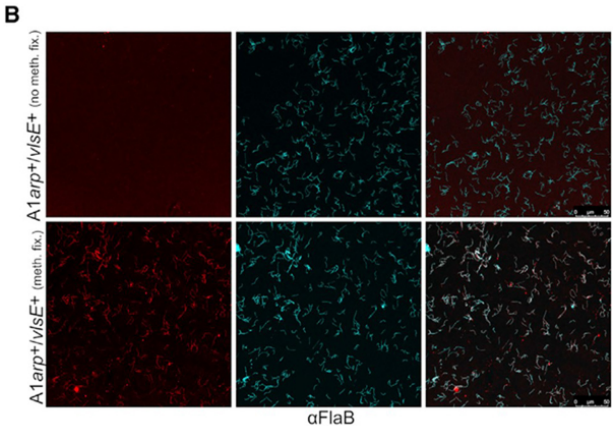
Physical State:	Lyophilized
Concentration:	1.2 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	100 µL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

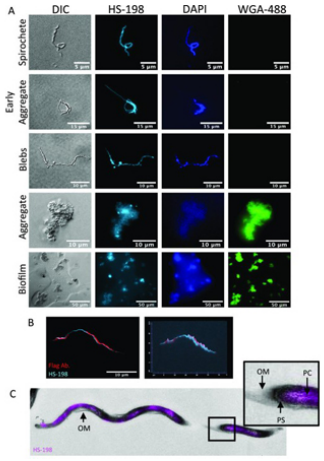
Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

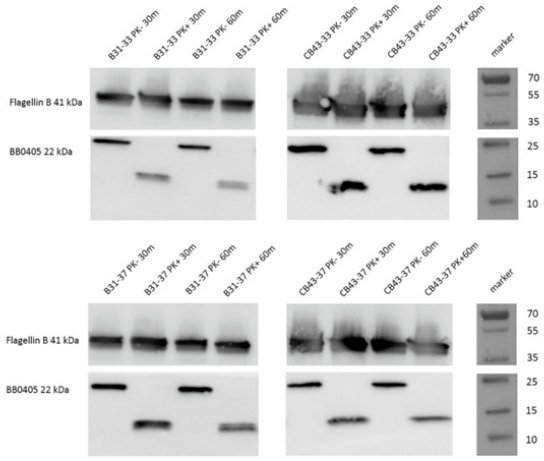
Images



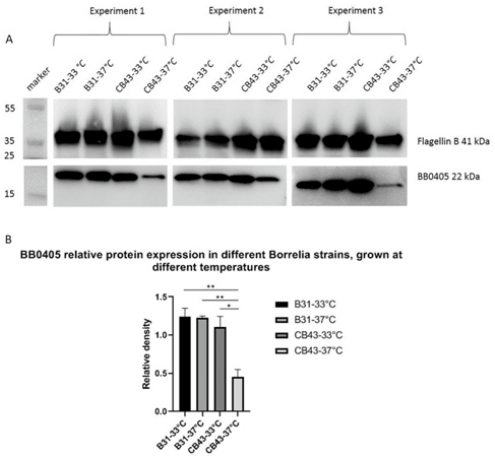
Immunofluorescence Microscopy
(B) A1arp+/vlsE+ spirochetes were probed with anti-FlaB antibodies (αFlaB) to verify outer membrane integrity during immunofluorescence. The anti-FlaB antibodies could access the periplasmic flagellar protein only when permeabilized with methanol first (bottom row). Each immunofluorescence assay was conducted three times with two independent sets of clones. The scale bar represents 50 µm. meth. fix., methanol fixation. Fig 2.
PMID: 32187539



Immunofluorescence Microscopy
HS-198 accumulation and localization in B. burgdorferi. (A) Fluorescent imaging of B. burgdorferi in spirochetes, blebs, and aggregates stained with HS-198 (HtpG), WGA-488 (extracellular matrix), and DAPI (DNA). Images shown are representative of n = 48. (B) Confocal images (100×) of HS-198 and flagellin antibody staining of a spirochete (n = 5). (C) Correlative light electron microscopy (CLEM) of HS-198 fluorescence overlaying an EM image of a B. burgdorferi spirochete. Outer membrane (OM), protoplasmic cylinder (PC), and periplasmic space (PS) are identified. Images shown are representative of n = 4 spirochetes with TEM sections of 65 nm. Fig 2. PMID: 33910962

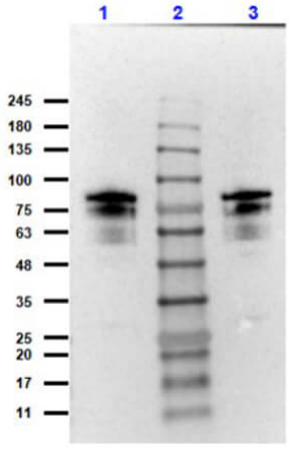


Western Blot
BB0405 is expressed on the surface of B. burgdorferi sensu stricto and B. afzelii. Viable spirochetes were incubated with (+) or without (−) proteinase K for 30 min and 60 min and processed for immunoblot analyses with antibodies against BB0405. Flagellin B antibodies served as a subsurface control. Fig 3. PMID: 33637813

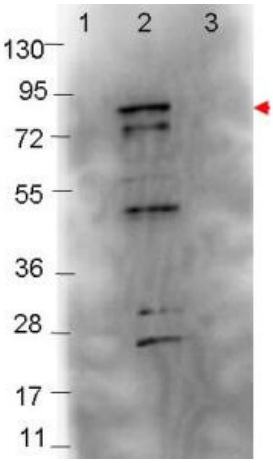


Western Blot

(A) Western blot showing expression of BB0405 in different B. burgdorferi sl strains (Borrelia burgdorferi strain B31 and Borrelia afzelii strain CB43) grown at different temperatures. A temperature of 33 °C, resembling (feeding) tick conditions, and 37 °C, resembling conditions in the mammalian host, was used to culture the spirochetes. Lysates of the in vitro cultured spirochetes were obtained, as described in the materials and method section, and subjected to Western blot. Three independent experiments were performed. The first experiment is displayed on the left, the second experiment in the middle and the third experiment is displayed on the right. The far left lane is the protein weight marker. Blots were loaded with 2.5 ug/well whole lysates of spirochetes and were cut in half just between the bands of Flagellin B (41 kDa) and BB0405 (22 kDa) to enable separate incubations. The blots displayed at the top are incubated with anti-flagellin rabbit IgG 1:1000 (as a loading control) and subsequently with secondary antibody anti-rabbit IgG-HRP 1:1000. Blots displayed at the bottom are incubated with pooled serum from mice vaccinated with recombinant BB0405 1:500 and subsequently with secondary antibody anti-mouse IgG-HRP 1:2000, respectively. Blot images were cropped (Image acquisition tools Microsoft Powerpoint). Imaging was performed using ImageQuant LAS 4000 and quantification using Image J (Wayne Rasband, National Institutes of Health, USA, Java 1.8.0_77(32-bit), http://imagej.nih.gov/ij). Full length blots are presented in Supplementary Fig. S1–S6.
(B) Protein expression of BB0405 as determined by Western blot in panel A was quantified and normalized against the relative density of loading control Flagellin B. Relative density was determined using ImageJ software (National Institute of Health). A quantitative comparison between samples on the same blot and within the same experiment were made. Statistical differences between groups were calculated using an unpaired parametric t-test. Error bars represent mean ± s.e.m. n.s.: $P > 0.05$. Fig 4. PMID: 33637813



Western Blot
Western Blot of Anti-Flagellin Antibody. Lane 1: Flagellin Control Protein (p/n 000-001-C14) Reduced [0.1µg]. Lane 2: Opal Prestained Molecular Weight Markers (p/n MB-210-0500). Lane 3: Flagellin Control Protein Non-Reduced [0.1µg]. Primary Antibody: Anti-Flagellin at 1.0µg/mL overnight at 4°C. Secondary Antibody: Goat anti-Rabbit IgG peroxidase (p/n 611-103-122) at 1:70,000 for 30 mins at RT. Block: BLockOut (p/n MB-073) for 1hrs at RT. Predicted MW: ~76.3kDa. Exposure: 2sec.



Western Blot
Western blot showing detection of 0.1 µg of recombinant Flagellin protein. Lane 1: Molecular weight markers. Lane 2: MBP-Flagellin fusion protein (arrowhead at expected MW: 76.3 kDa). Lane 3: MBP alone. Protein was run on a 4-20% gel, then transferred to 0.45 µm nitrocellulose. After blocking with 1% BSA-TTBS (p/n MB-013, diluted to 1X) overnight at 4°C, primary antibody was used at 1:1000 at room temperature for 30 min. HRP-conjugated Goat-Anti-Rabbit (p/n 611-103-122) secondary antibody was used at 1:40,000 in MB-070 blocking buffer and imaged on the VersaDoc™ MP 4000 imaging system (Bio-Rad).

References

- Klouwens, M J et al. Investigating BB0405 as a novel Borrelia afzelii vaccination candidate in Lyme borreliosis. *Scientific Reports* (2021)
- Sell MG et al. Visualizing Borrelia burgdorferi Infection Using a Small-Molecule Imaging Probe. *J Clin Microbiol.* (2021)
- Lone A et al. The Borrelia burgdorferi VlsE Lipoprotein Prevents Antibody Binding to an Arthritis-Related Surface Antigen. *Cell Rep.* (2020)

Disclaimer

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