

Product Datasheet

Aflatoxin B1 Antibody (6A10) - BSA Free NB600-443SS

Unit Size: 0.025 ml

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

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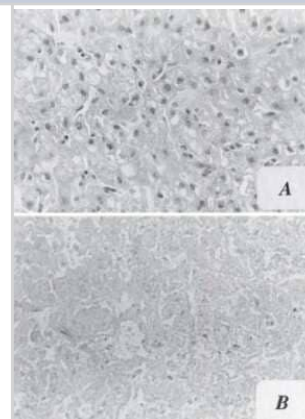
NB600-443SS

Aflatoxin B1 Antibody (6A10) - BSA Free

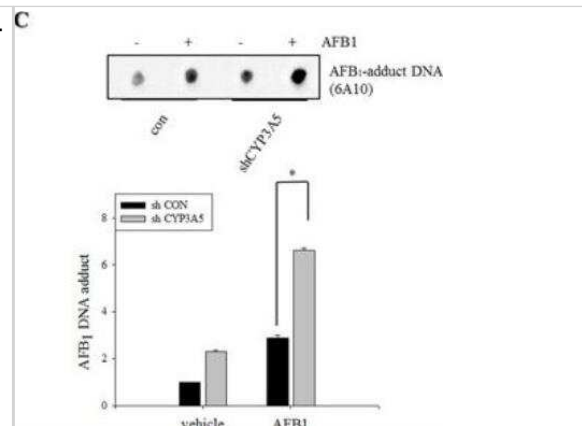
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	6A10
Preservative	0.1% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Product Description	
Host	Mouse
Species	Human, All Species
Immunogen	Aflatoxin B1 DNA (against the midazole ring-opened persistent form of the major N-7 guanine adduct of AFB1).
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Dot Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen
Recommended Dilutions	Western Blot 1:500-1:2000, ELISA 5 ug/ml, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:100. Use reported in scientific literature (PMID 24114584), Immunohistochemistry-Paraffin reported in scientific literature (PMID 24391771), Immunohistochemistry-Frozen, Dot Blot reported in scientific literature (PMID 19524575)

Images

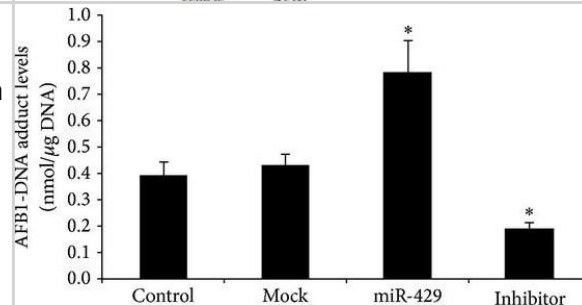
Immunohistochemistry: Aflatoxin B1 Antibody (6A10) [NB600-443] - Staining of positive (A) and negative (B) tumor tissues for AFB₁-DNA adducts. Photos courtesy of Dr. Regina Santella.



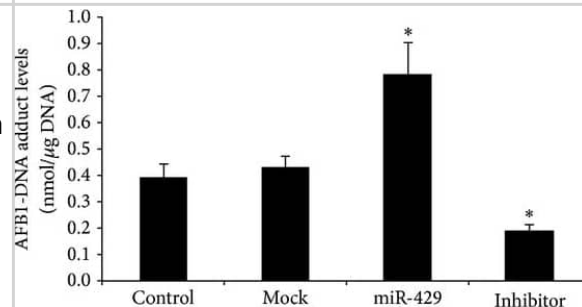
Dot Blot: Aflatoxin B1 Antibody (6A10) [NB600-443] - HCT-8 cells stably-transfected with empty vector or shCYP3A5 were treated with DMSO or 10 mM AFB1 for 72 h. AFB1 DNA adduct (6A10) were detected using immunodot-blot assay. DNA adduct measured by multi gauge software (bottom panel), respectively. Image collected and cropped by CiteAb from the following publication (oncotarget.com/article/8914/text/), licensed under a CC-BY license.



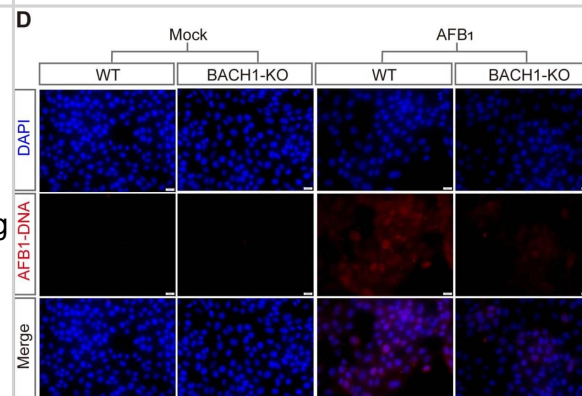
AFB1-DNA adducts formation in AFB1-treated SMMC-7721 cells with overexpression of miR-429 (see Section 2). Levels of AFB1-DNA adducts were tested using comparative ELISA. Data were analyzed from three independent tests using one-way ANOVA with Bonferroni corrections. *P < 0.05.



ELISA: Aflatoxin B1 Antibody (6A10) - BSA Free [NB600-443] - AFB1-DNA adducts formation in AFB1-treated SMMC-7721 cells with overexpression of miR-429 (see Section 2). Levels of AFB1-DNA adducts were tested using comparative ELISA. Data were analyzed from three independent tests using one-way ANOVA with Bonferroni corrections. *P < 0.05. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/24204382/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



BACH1 knockout cells exhibit higher resistance to aflatoxin B1. (A) Western blot analysis of BACH1 expression in WT and KO cells. (B) Representative images of WT and BACH1-KO PK-15 cells challenged with 2 μg/mL AFB1 for 48 h. Scale bar, 100 μM. (C) The IC50 values for AFB1 in WT and BACH1-KO cells determined by CCK-8 assays. (D,E) Immunofluorescence staining of AFB1-induced DNA adduct formation in WT and BACH1-KO cells; relative fluorescence intensity calculated using ImageJ software. Scale bar, 20 μM. (F) Enhanced resistance to AFM1, AFG1, and F-2 in BACH1-KO cells. WT and BACH1-KO cells were treated with AFM1 (at 0.5 μg/mL and 1 μg/mL), AFG1 (at 8 μg/mL and 10 μg/mL), and F-2 (at 20 μg/mL and 30 μg/mL) for 36 h. Cell viability was measured with CCK-8 assays. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. p values were determined with two-tailed Student's t-tests. AFB1, aflatoxin B1; AFG1, aflatoxin G1; F2, zearalenone; WT, wild-type; KO, knockout. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36139865/>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhang J, Hu S, Zhao C et al. Genome-Scale CRISPR Knockout Screening Identifies BACH1 as a Key Regulator of Aflatoxin B(1)-Induced Oxidative Damage Antioxidants (Basel) 2022-09-10 [PMID: 36139865]

Xu C, Cheng S, Chen K et al. Sex Differences in Genomic Features of Hepatitis B-Associated Hepatocellular Carcinoma With Distinct Antitumor Immunity Cellular and Molecular Gastroenterology and Hepatology 2023-01-01 [PMID: 36272708]

Huang L, Long Q, Su Q et al. Aflatoxin B1-DNA adducts modify the effects of post-operative adjuvant transarterial chemoembolization improving hepatocellular carcinoma prognosis Explor Target Antitumor Ther 2023-08-31 [PMID: 37711588]

Gramantieri L, Gnudi F, Vasuri F et al. Aflatoxin B1 DNA-Adducts in Hepatocellular Carcinoma from a Low Exposure Area Nutrients 2022-04-15 [PMID: 35458213] (IHC-P)

Zhu Q, Ma Y, Liang J Et al. AHR mediates the aflatoxin B1 toxicity associated with hepatocellular carcinoma Signal transduction and targeted therapy 2021-08-09 [PMID: 34373448] (WB, CoIP, Human)

Ates MB, Ortatatlı M The effects of Nigella sativa seeds and thymoquinone on aflatoxin phase-2 detoxification through glutathione and glutathione-S-transferase alpha-3, and the relationship between aflatoxin B1-DNA adducts in broilers Toxicol : official journal of the International Society on Toxinology 2021-04-15 [PMID: 33581172] (IHC-P)

Wang L, He L, Zeng H et al. Low-dose microcystin-LR antagonizes aflatoxin B1 induced hepatocarcinogenesis through decreasing cytochrome P450 1A2 expression and aflatoxin B1-DNA adduct generation Chemosphere 2020-06-01 [PMID: 32045972] (IHC-P, Rat, Mouse)

Kim J, Park SH, Do KH et al. Interference with mutagenic aflatoxin B1-induced checkpoints through antagonistic action of ochratoxin A in intestinal cancer cells: a molecular explanation on potential risk of crosstalk between carcinogens. Oncotarget. 2016-06-28 [PMID: 27119350] (Cytometric Bead Assay Standard, Human)

Yang X, Zhang Z, Wang X et al. Cytochrome P450 2A13 enhances the sensitivity of Human bronchial epithelial cells to aflatoxin B1-induced DNA damage. Toxicol Appl Pharmacol 2013-04-18 [PMID: 23602888] (ICC/IF)

Details:

Using the HRP conjugated version of NB600-443, catalog number NB600-443H.

Zhang Z, Lu H, Huan F et al. Cytochrome P450 2A13 mediates the neoplastic transformation of human bronchial epithelial cells at a low concentration of aflatoxin B1. Int J Cancer 2013-09-30 [PMID: 24114584] (ICC/IF)

Details:

Using the HRP conjugated version of NB600-443, catalog number NB600-443H.

Qureshi H, Hamid SS, Ali SS et al. Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier Med. Mycol. [PMID: 25851265] (Cytometric Bead Assay Standard)

Details:

Citation using the HRP form of this antibody.

Liu Yi-Xiao, Long Xi-Dai, Xi Zhi-Feng et al. MicroRNA-24 modulates aflatoxin B1-related hepatocellular carcinoma prognosis and tumorigenesis. Biomed Res Int. 2014-04-08 [PMID: 24800232] (ELISA, Human)

More publications at <http://www.novusbio.com/NB600-443>

Procedures

Serum protocol for Aflatoxin B1 Antibody (NB600-443)

Competitive ELISA

Coating of Plates

DNA coating: DNA is dissolved in PBS at appropriate concentration. 0.1 ml is added/well and plates put in 37 degrees Celsius incubator to evaporate overnight. Alternatively, plates can be coated with a 2-fold higher concentration of DNA for 2 hrs at 37 degrees Celsius then used. Column 1 is not coated. These well will not be used for the assay (no blocking, no antibody and no secondary antibody) but will have substrate added for blanking the reader. Plates are stored in the refrigerator.

Protein coating: Proteins are dissolved in PBS at the appropriate concentration. 0.1 ml is added/well and plates put in 37 degrees Celsius incubator to evaporate overnight. Column 1 is again not coated. Plates are stored in the refrigerator.

An alternate protein coating condition is to dissolve the protein in 0.1 M sodium carbonate buffer pH 9.6. 0.1 ml is added/well and the plates are refrigerated for several hours or overnight. They cannot be used after 3 days.
1 M solution 1.59 g Na₂CO₃ + 2.93 g NaHCO₃/100ml

Assay

1. Label assay sheet and determine which rows are to be used. Row 1 (A-H) is not used; it will be used to blank the spectrophotometer. Avoid using the outer rows if possible (i.e. 12A-H, H 1-12 and A 1-12).
2. Wash plate with wash buffer containing PBS-Tween and NaN₃ 3 x on each side (right side up and upside down). Shake out onto paper towel.
3. Add 0.2 ml/well of 1% FCS in wash buffer to block non specific binding. Solution of FCS should be made fresh.
4. Incubate 1 hr.
5. Preparation of inhibitor series (during incubation of plate with FCS). Calculate appropriate concentrations to give desired fmol/well=fmol/0.05 ml. Make serial dilutions by adding PBS or CT DNA to tubes followed by competitor.
6. Prepare antibody in 1% FCS washing buffer.
7. At end of incubation period, shake out solution from plate and tap onto paper towel to dry.
8. Add 0.05 ml of competitor to each well followed by 0.05 ml of diluted antibody. Be sure to run all controls including zero (no competitor), minus Ab (no antigen specific antibody but secondary antisera) and positive and negative controls.
9. Incubate for 90 min at 37 degrees Celsius.
10. Wash the plate with washing buffer 3 times on each side. Tap onto paper towels.
11. Secondary antisera - Use goat anti-mouse IgG-alkaline phosphatase for monoclonals and anti rabbit for polyclonals. Dilute as appropriate and add 0.1 ml/well.
12. Incubate for 90 min at 37 degrees Celsius.
13. Wash with wash buffer 3 x each side. Tap onto paper towel.
14. Wash plate 2 times with 0.01 M diethanolamine using the was bottle and covering the well completely each time. Tap onto paper towel. This step removes phosphate buffer which inhibits alkaline phosphatase activity.
15. Prepare the substrate - 2 tablets 95 mg/tablet) Sigma 104 in 10 ml 1 M diethanolamine, pH 8.6. Final

concentration 1 mg/ml. Avoid physical contact of skin with the tablets since skin contains alkaline phosphatase. Add 0.1 ml/well

16. Incubate at 37 degrees Celsius and read absorbance at 405 nm. The absorbance of the 0 fmol standard should be between 0.5 and 1. Values above 2 are not usable since the reader may not be linear in this range.

Rinse water - One liter of H₂O + 2 ml 10% NaN₃

Wash buffer - One liter of 1 x PBS + 500 ul Tween 20 + 2 ml 10% NaN₃

Blocking buffer - Wash buffer + 1% FCS





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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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