Product Datasheet

Ethenoadenosine Antibody (1G4) - BSA Free
NB600-442

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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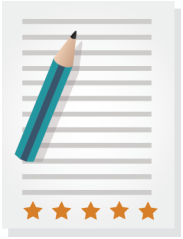
Updated 10/23/2024 v.20.1



NB600-442

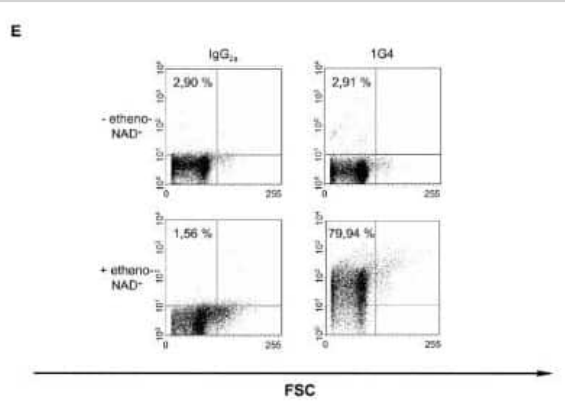
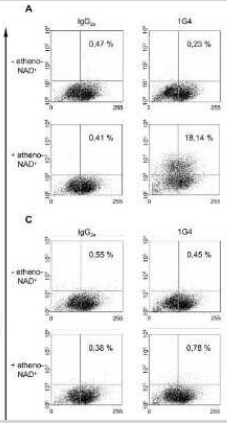
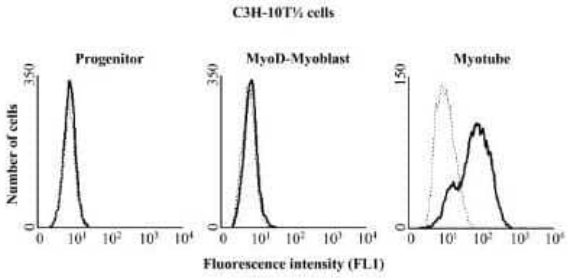
Ethenoadenosine Antibody (1G4) - BSA Free

Product Information	
Unit Size	0.1 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	1G4
Preservative	0.1% Sodium Azide
Isotype	IgG2 Lambda
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Species	All Species
Specificity/Sensitivity	This is specific for ethenoadenosine and ethenodadenosine.
Immunogen	Ethenoadenosine

Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 1:800, Flow Cytometry 1:10-1:1000. Use reported in scientific literature (PMID 9774627), ELISA 1:100-1:2000, Immunocytochemistry/ Immunofluorescence 1:50-1:100, Immunoprecipitation 1:10-1:500, CyTOF-ready
Application Notes	This antibody is CyTOF ready.

Images

Flow Cytometry: Ethenoadenosine Antibody (1G4) [NB600-442] - Chicken erythrocytes (1% v/v), isolated by density gradient centrifugation, were transiently transfected with a Flag-tagged chicken ART4 containing plasmid or the empty plasmid (pSecTag B), and incubated at 37C in the presence or absence of 200 uM etheno-NAD+ for 30 min. After washing, cells were stained with an etheno-adenosine specific antibody (1G4) or an anti-Flag antibody and the respective isotype control stained with the 1G4 antibody. Image collected and cropped by CiteAb from the following publication (https://bmcmolbiol.biomedcentral.com/articles/10.1186/1471-2199-9-86), licensed under a CC-BY license.	
Flow Cytometry: Ethenoadenosine Antibody (1G4) [NB600-442] - Detection of ADP-ribosylated proteins by flow cytometry. C-33A cells (1 x 10^7/mL), transiently transfected with a Flag-tagged chicken ART4 containing plasmid, were incubated at 37C in the presence or absence of 200 uM etheno-NAD+ for 30 min. After washing, cells were stained with an etheno-adenosine specific antibody (1G4). Image collected and cropped by CiteAb from the following publication (https://bmcmolbiol.biomedcentral.com/articles/10.1186/1471-2199-9-86), licensed under a CC-BY license.	
Flow Cytometry: Ethenoadenosine Antibody (1G4) [NB600-442] - Etheno-ADP-ribosylation of cell-surface proteins on C3H-10T1/2 cells. C3H-10T 1/2 cells at different stages of differentiation (progenitors, MyoD-myoblasts, myotubes) were incubated for 30 minutes in the absence (dashed lines) or presence (solid lines) of 100 uM etheno-NAD. Cells were then washed, incubated with the primary monoclonal antibody 1G4, stained with a FITC-conjugated goat-anti-mouse secondary antibody and subjected to FACS analysis. Data show one representative experiments out of three. Image collected and cropped by CiteAb from the following publication (https://bmcmolbiol.biomedcentral.com/articles/10.1186/1471-2199-9-91), licensed under a CC-BY license.	

Publications

Brown EM, Arellano-Santoyo H, Temple ER Et al. Gut microbiome ADP-ribosyltransferases are widespread phage-encoded fitness factors Cell host & microbe 2021-08-10 [PMID: 34403684]

Rack JGM, Zorzini V, Zhu Z et al. Viral macrodomains: a structural and evolutionary assessment of the pharmacological potential Open Biol 2020-11-01 [PMID: 33202171] (WB)

Koch-Nolte F, Reyelt J, Schossow B et al. Single domain antibodies from llama effectively and specifically block T cell ecto-ADP-ribosyltransferase ART2 2 in vivo. FASEB J. [PMID: 17575259] (ICC/IF, Mouse)

Details:
Citation using the FITC form of this antibody.

Liao, SD, Puro, DG. NAD+-induced vasotoxicity in the pericyte-containing microvasculature of the rat retina: effect of diabetes. Invest Ophthalmol Vis Sci;47(11):5032-8. 2006-11-01 [PMID: 17065524] (ICC/IF, Rat)

Ohlrogge, W et al. Generation and characterization of ecto-ADP-ribosyltransferase ART2.1/ART2.2-deficient mice. Mol Cell Biol. 22(21):7535-42. 2002-11-01 [PMID: 12370300]

Ablamunits V, Bridgett M, Duffy T, Haag F, Nissen M, Koch-Nolte F, Leiter H. Changing patterns of cell surface mono (ADP-ribosyl) transferase antigen ART2.2 on resting versus cytopathically-activated T cells in NOD/Lt mice. Diabetologia;44(7):848-58. 2001-07-01 [PMID: 11508269] (FLOW, Mouse)

Davis RE, Mysore V, Browning JC, Hsieh JC, Lu QA, Katsikis PD. In situ staining for poly(ADP-ribose) polymerase activity using an NAD analogue. J Histochem Cytochem;46(11):1279-89. 1998-11-01 [PMID: 9774627] (ICC/IF, FLOW, Human)

Young, TL, Santella, RM. Development of techniques to monitor for exposure to vinyl chloride: monoclonal antibodies to ethenoadenosine and ethenocytidine. Carcinogenesis;9(4):589-92. 1988-04-01 [PMID: 3356066]

Aswad F, Kawamura H, Dennert G. High sensitivity of CD4+CD25+ regulatory T cells to extracellular metabolites nicotinamide adenine dinucleotide and ATP: a role for P2X7 receptors. J Immunol;175(5):3075-83. 2005-09-01 [PMID: 16116196] (FLOW)

Krebs, C et al. Flow cytometric and immunoblot assays for cell surface ADP-ribosylation using a monoclonal antibody specific for ethenoadenosine. Anal Biochem;314(1):108-15. 2003-03-01 [PMID: 12633608] (FLOW, WB, ICC/IF, Mouse, Human)

Friedrich M, Bohlig L, Kirschner RD, EngelK, Hauschildt S. Identification of two regulatory binding sites which confer myotube specific expression of the mono-ADP-ribosyltransferase ART1 gene. BMC Mol Biol;9:91. 2008-10-21 [PMID: 18939989] (FLOW)

Grahnert A, Richter S, Siegert F, Berndt A, Hauschildt S. The orthologue of the acatalytic mammalian ART4 in chicken is an arginine-specific mono-ADP-ribosyltransferase. BMC Mol Biol;9:86. 2008-10-14 [PMID: 18854029] (WB, FLOW, Human, Chicken)

More publications at http://www.novusbio.com/NB600-442

Procedures

Serum protocol for Ethenoadenosine Antibody (NB600-442)

ICoating 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 Na_2CO_3 + 2.93 g $NaHCO_3$/100ml

II 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 NaN_3 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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Products Related to NB600-442

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NB600-442B	Ethenoadenosine Antibody (1G4) [Biotin]

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