Anti-8-Nitro-cGMP

Background

8-Nitroguanosine is a nitrated nucleic acid which is formed from the reactions of guanosine with peroxynitrite, myeloperoxidase, nitrite, and peroxide. It is known that the nitration of guanine is enhanced in virus infection[1, 2], bacterial infection[3, 4], inflammatory disease[5] , cancers[5] , and diseases associated with smoking[6] . 8-Nitroguanosine is thought to be one of the makers of DNA damage caused by oxidative stress. Cyclic GMP (cGMP) is a second messenger that activates protein kinase G. 8-Nitro-cGMP (nitrated cGMP) has been identified as a nitrated cGMP formed in vivo[3] . 8-Nitro-cGMP can act as a unique second messenger distinct from cGMP to induce antioxidative adaptive responses[3, 7-9]. Mode of actions of 8-nitro-cGMP mainly relies on its adduction to protein cysteine residues called “protein S-guanylation”, as a posttranslational modification.

Product type	Primary Antibody
Immunogen	8-Nitro-cGMP-conjugated bovine serum albumin
Raised in	Mouse
Clone number	1G6
Isotype	IgG1
Source	Ascites
Purification	Ion-Exchange Chromatography
Buffer	10 mM Tris-HCl (pH 7.4) + 50 mM NaCl (no preservatives added)
Concentration	1 mg/mL
Volume	50 ug
Label	Unlabeled
Specificity	8-Nitro-cGMP
Storage	Store at -70℃ Aliquot to avoid cycles of freeze/thaw.

Recommended Dilutions	Immunocytochemistry, 1 ug/mL in Can get signal solution I Other applications have not been tested or not reactive. Optimal dilutions/concentrations should be determined by the end user.

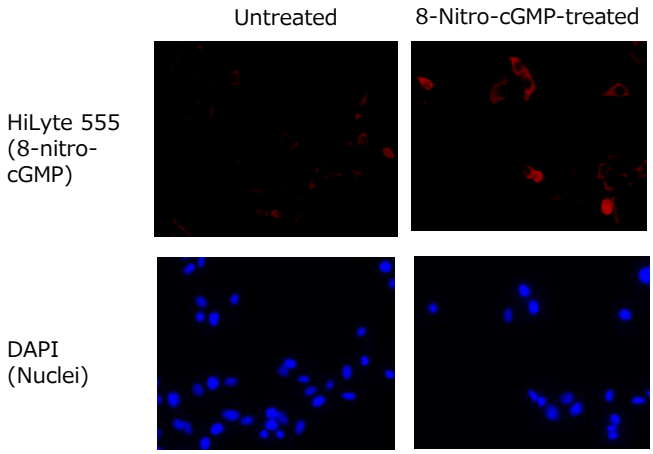

Fig 1. Immunocytochemistry of 8-nitro-cGMP in C6 cells treated with authentic 8-nitro-cGMP.
C6 cells (0.25 x 10^5 cells in 0.5 mL/well containing circle glass) were treated with 8-nitro-cGMP(100 μM) or untreated for 3 h in FBS-free DMEM.
Cells were fixed with Zamboni solution (4% paraformaldehyde and 10 mM picric acid in 0.1 M phosphate buffer, pH 7.4) at room temperature (RT) for 1 h. After washing with PBS, the preparations were permeabilized with PBS containing 0.5% TritonX-100 at RT for 15 min, washed, and incubated in BlockAce at RT for 1 h .
After washing with TBST three times, the preparations were incubated with 1G6 (1 μg/mL in Can get signal solution I) at 4 °C over night. After washing with TBST three times, the preparations were incubated with goat anti-mouse IgG (H+L), highly cross-adsorbed, HiLyte™ Fluor 555-labeled (AnaSpec, Inc, San Jose, CA) (0.2 μg/mL ,1:5000 in Can get signal solution II) at RT for 1 h. After washing with TBST three times and with distilled water one time, the preparations were mounted on glass slides using Prolong® Diamond Antifade Mountant with DAPI (Invitrogen; Carlsbad, CA).

Fig 2. Immunocytochemistry of 8-nitro-cGMP formed in immunologically stimulated C6.
C6 cells (0.25 x 10^5 cells in 0.5 mL/well containing circle glass) were treated with LPS 10 μg/mL + Cytokines (10 ng/mL IL-1β, 100 U/mL TNF-α and 100 U/mL IFN-γ) (all cytokines from R & D Systems) for 30 h.
Cell fixation and immunocytochemistry protocols were same as that for Fig. 1.

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