



Next Generation Fluorescence Imaging

Smart Sensor Solutions

General Information

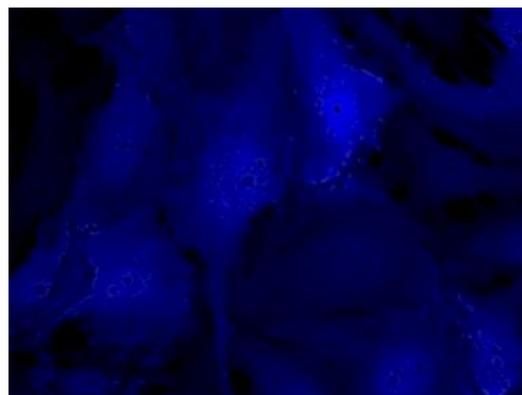
This recombinant human adenovirus type 5 expresses C-geNOP^{mut} (Δ C-geNOP AAV5) under the control of a CMV promoter.

Contents and Storage

Recombinant adenovirus is supplied in liquid form at indicated titer. Store at -80°C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer immediately. DO NOT FREEZE AND THAW REPEATEDLY.

Description

C-geNOP^{mut} is a cyan fluorescent-based probe serving as a negative control for the nitric oxide (NO•) sensitive sensor C-geNOP within the cytosol of intact mammalian cells. Infection of recombinant adenovirus encoding C-geNOP^{mut} represents as an alternative for difficult plasmid transfection into mammalian cells with almost 100 % efficiency of gene delivery in most cell types. Δ C-geNOP AAV5 can be added directly to cells in culture medium in the presence or absence of serum. Usually cells express high amounts of C-geNOP^{mut} 24 – 48 hours after cell infection. Standard optical filters for CFP imaging should be used. Δ C-geNOP AAV5 can be used for subcloning C-geNOP^{mut}.



C-geNOP^{mut} expression in adenovirus infected Vascular Smooth Muscle Cells (VSMC).

Guideline for the infection of Δ C-geNOP AAV5

The amount of adenovirus cell receptors largely varies among different cell types and an optimal concentration needs to be determined for the cell line of choice. An appropriate amount of virus of infected cells is important for the outcome of experiments. The goal is to achieve a 100 % infection efficiency without causing cytotoxicity. Due to the emitting fluorescence of C-geNOP^{mut} expressing cells this adenovirus is suitable for pilot testing the appropriate amount of virus for an individual mammalian cell type. We recommend to use different virus concentrations ranging from 1 to 1000 MOI (multiplicity of infection) and to determine the optimal amount by monitoring the cyan fluorescence 24, 48 and 72 hours after infection. Most cell types like HEK293, HeLa or smooth muscle cells range between 1 and 100 MOI, but up to 1000 MOI may be used for lymphoid or endothelial cell lines.

NOTE: For a correct negative control of C-geNOP, Δ C-geNOP needs to be treated with the non-toxic iron(II) loading buffer (<http://www.ngfi.eu/product/ironii-booster-solution/>) for 20 minutes before imaging experiments.

References

Eroglu E. (2016) "Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics" (<http://www.nature.com/ncomms/2016/160204/ncomms10623/pdf/ncomms10623.pdf>)

Opelt M. (2016) "Formation of Nitric Oxide by ALDH-2 Is Necessary and Sufficient for Vascular Bioactivation of Nitroglycerin" (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5104933/pdf/zbc24076.pdf>)

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