



Next Generation Fluorescence Imaging

Smart Sensor Solutions

General Information

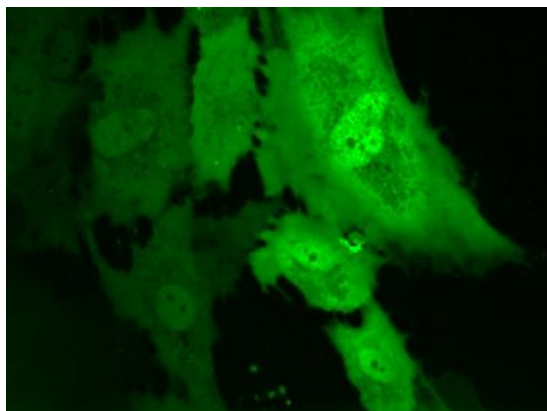
This recombinant human adenovirus type 5 expresses Δ G-geNOP (G-geNOP AV5) under the control of a CMV promoter.

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

Δ G-geNOP is a green fluorescent genetically encoded biosensor serving as a negative control of G-geNOP capable for specific detection of intracellular (cytosolic) nitric oxide ($\text{NO}\bullet$) dynamics within intact living mammalian cells. Infection of recombinant adenovirus encoding Δ G-geNOP represents as an alternative for difficult plasmid transfection into mammalian cells with almost 100 % efficiency of gene delivery in most cell types. Δ G-geNOP AV5 can be added directly to cells in culture medium in the presence or absence of serum. Usually cells express high amounts of Δ G-geNOP 24 – 48 hours after cell infection. Standard optical filters for GFP imaging should be used. Δ G-geNOP AV5 can be used for subcloning Δ G-geNOP.



Δ G-geNOP expression in adenovirus infected Human Umbilical Vein Endothelial Cells (HUVEC).

Guideline for the infection of Δ G-geNOP AV5

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 Δ G-geNOP 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 green 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: In order to supply the $\text{NO}\bullet$ -binding domain of G-geNOP/ Δ G-geNOP with sufficient iron(II) cells need to be treated with the non-toxic iron(II) loading buffer (<http://www.ngfi.eu/product/ironii-booster-solution/>) for at least 10 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>)

Charoensin S. (2017) "Intact mitochondrial Ca^{2+} uniport is essential for agonist-induced activation of endothelial nitric oxide synthase (eNOS)" (<http://www.sciencedirect.com/science/article/pii/S0891584916310851>)

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