General Information

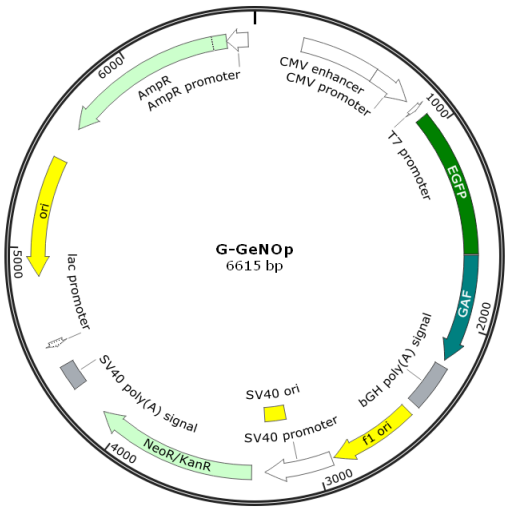
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial use.

G-geNOp vector

This vector has not been completely sequenced. All provided information regarding the vector composition was compiled using the information from published literature, other sources together with partial sequences obtained by NGFI.

Vector description

G-geNOp is a green fluorescent-based probe for imaging nitric oxide (NO•) within the cytosol of intact mammalian cells. In order to express G-geNOp in cells of interest, 20 µg of purified endotoxin-free plasmid DNA coding for G-geNOp is provided. The plasmid coding for G-geNOp represents a mammalian expression vector with a strong viral promotor. For plasmid amplification in E.coli ampicillin should be used. 1 – 1.5 µg DNA is required for cell transfection in a single well of a standard 6-well dish following standard transfection procedures. Usually cells express high amounts of G-geNOp 24 – 48 hours after cell transfection. Standard optical filters for GFP imaging should be used. The vector can be also used as a source of G-geNOp coding sequence.



Expression in mammalian cells

G-geNOp vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive G-geNOp expression in eukaryotic cells.

Propagation in E. coli

Suitable host strains for propagation in E. coli include DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to ampicillin (100 µg/ml) to E. coli hosts.

NOTE: In order to supply the NO•-binding domain of 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)

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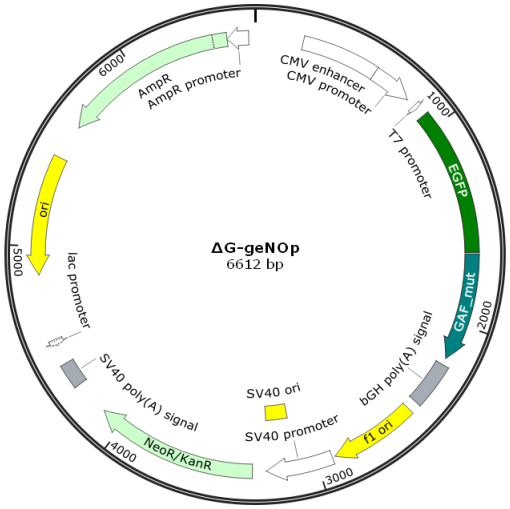
ΔG-geNOp vector

This vector has not been completely sequenced. All provided information regarding the vector composition was compiled using the information from published literature, other sources together with partial sequences obtained by NGFI.

Vector description

ΔG-geNOp is a green fluorescent-based probe serving as a negative control for the nitric oxide (NO•) sensitive sensor G-geNOp within the cytosol of intact mammalian cells. In order to express ΔG-geNOp in cells of interest, 20 µg of purified endotoxin-free plasmid DNA coding for ΔG-geNOp is provided. The plasmid coding for ΔG-geNOp represents a mammalian expression vector with a strong viral promotor. For plasmid amplification in E.coli ampicillin should be used. 1 – 1.5 µg DNA is required for cell transfection in a single well of a standard 6-well dish following standard transfection procedures. Usually cells express high amounts of ΔG-geNOp 24 – 48 hours after cell transfection. Standard optical filters for GFP imaging should be used. The vector can be also used as a source of ΔG-geNOp coding sequence.



Expression in mammalian cells

ΔG-geNOp vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive ΔG-geNOp expression in eukaryotic cells.

Propagation in E. coli

Suitable host strains for propagation in E. coli include DH5alpha, HB101, XL1-Blue, and other general purpose strains. The vector confers resistance to ampicillin (100 µg/ml) to E. coli hosts.

NOTE: For a correct negative control of G-geNOp, ΔG-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)

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Instruction Sheet: Iron-buffer pre-incubation of cells expressing geNOps

0. Aim of this instruction is to achieve uniformity of the performance of measuring nitric oxide (NO) in single cells, using genetically encoded nitric oxide probes (geNOps). geNOps expressing cells need to be pre-incubated prior to imaging experiments in order to restore Fe(II) within the NO•-binding domain of the NO-probes. Usually, incubation of cells for 20 minutes with the *booster solution* leads to full activation of the sensors. It is recommended to let cells equilibrate after iron-loading for at least 1 hour. If you observe deviations in your measurement results check the protocol and announce it immediately. Never use stale solutions for your experiments. For trouble-shooting, contact our technical service team support@ngfi.eu.

1. Protocol:

- Replace medium with undiluted iron(II) booster solution at room temperature
- Keep cells in iron(II) booster solution for 20 minutes at room temperature in the dark
- Replace iron(II) booster solution with experimental buffer, cell storage or culture media
- Let cells equilibrate for at least one hour prior to 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)