General Information

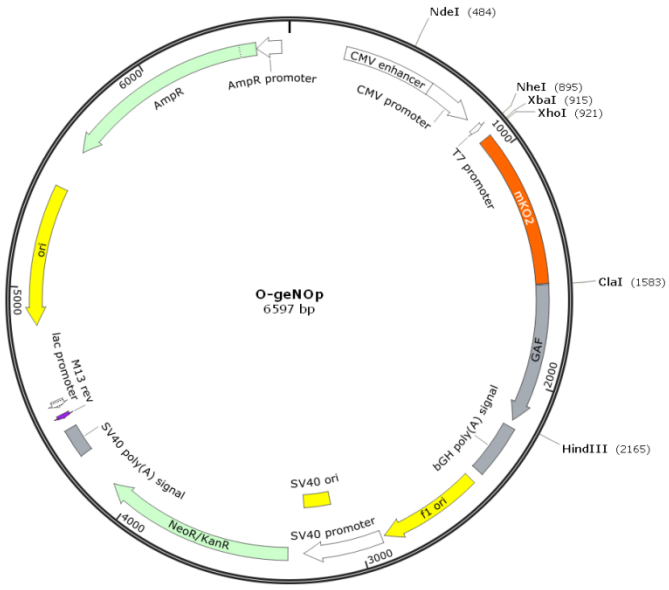
This product is non-toxic, non-contagious and is not intended for human use. The plasmid DNA in the package is synthetic and only for research and development purposes. It does not present any danger for humans, animals or the environment. The product is only for in vitro studies and is not for commercial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

O-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

O-geNOp is an orange fluorescent-based probe for imaging nitric oxide (NO•) within the cytosol of intact mammalian cells. In order to express O-geNOp in cells of interest, 20 µg of purified endotoxin-free plasmid DNA coding for O-geNOp is provided. The plasmid coding for O-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 O-geNOp 24 – 48 hours after cell transfection. Standard optical filters for OFP imaging should be used. The vector can be also used as a source of O-geNOp coding sequence. Flanking restriction sites are convenient for excision of O-geNOp sequence and its further insertion into other expression vectors of choice.



Expression in mammalian cells

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