General Information

This product is non-toxic, non-contagious and is not intended for human use. Human embryonic kidney cells (HEK293) stably expressing G-geNOp are delivered frozen in a cryovial. One vial contains ~ 10^7 cells and is only for research and development purposes. The product is only for in vitro studies and is not for commercial use.

Product description

G-geNOp is a green fluorescent-based probe for imaging nitric oxide (NO•) within the cytosol of HEK293 cells. HEK293 cells stably transfected with G-geNOp do not express any nitric oxide synthase (NOS) isoforms and hence cannot form NO itself. However this cell model is suitable for e.g. NOS studies. Therefor the NOS isoform of choice needs to be transfected into G-geNOp stably expressing HEK293 cells by any known transfection method. Usually cells express high amounts of G-geNOp. Standard optical filters for GFP imaging should be used. The cDNA of these sensor cells can be also used as a source of G-geNOp coding sequence.

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 20 minutes before imaging experiments.

Culture method

As base medium for HEK293 cells stably transfected with G-geNOp Dulbecco's Modified Eagle's Medium (#D5523, Sigma Aldrich, Vienna) is recommended. To make the complete growth medium, add fetal bovine serum to a final concentration of 10 %. For thawing place the frozen cells immediately into a 37°C water bath for < 1 minute by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial. Transfer the thawed cells into a centrifuge tube with 10 ml of pre-warmed complete growth medium. Then centrifuge the cell suspension at ~ 600 x g for 5 minutes. Aseptically decant the supernatant without disturbing the cell pellet and gently resuspend the cells in 10 ml of complete growth medium. We recommend transferring the cell suspension in a standard 100 mm dish for the first passage and keeping the cells in a humidified incubator (37 °C, 5% CO_2) until a cell growth density of 80 %. Re-fresh culture medium every 2-3 days.

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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