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)