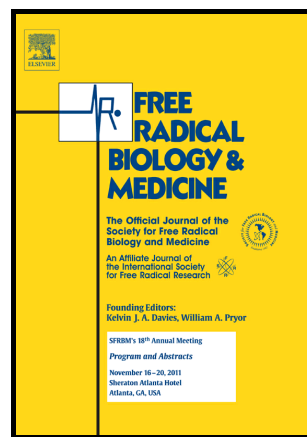


# Author's Accepted Manuscript

Flow cytometric HyPer-based assay for hydrogen peroxide

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## Abstract

HyPer is a genetically encoded fluorogenic sensor for hydrogen peroxide which is generally used for the ratiometric imaging of H<sub>2</sub>O<sub>2</sub> fluxes in living cells. Here, we demonstrate the advantages of HyPer-based ratiometric flow cytometry assay for H<sub>2</sub>O<sub>2</sub>, by using K562 and human mesenchymal stem cell lines expressing HyPer. We show that flow cytometry analysis is suitable to detect HyPer response to submicromolar concentrations of extracellularly added H<sub>2</sub>O<sub>2</sub> that is much lower than concentrations addressed previously in the other HyPer-based assays (such as cell imaging or fluorimetry). Suggested technique is also much more sensitive to hydrogen peroxide than the widespread flow cytometry assay exploiting H<sub>2</sub>O<sub>2</sub>-reactive dye H<sub>2</sub>DCFDA and, contrary to the H<sub>2</sub>DCFDA-based assay, can be employed for the kinetic studies of H<sub>2</sub>O<sub>2</sub> utilization by cells, including measurements of the rate constants of H<sub>2</sub>O<sub>2</sub> removal. In addition, flow cytometry multi-parameter ratiometric measurements enable rapid and high-throughput detection of endogenously generated H<sub>2</sub>O<sub>2</sub> in different subpopulations of HyPer-expressing cells. To sum up, HyPer can be used in multi-parameter flow cytometry studies as a highly sensitive indicator of intracellular H<sub>2</sub>O<sub>2</sub>.

Graphical abstract

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