NADPH oxidase activity: Spectrophotometric determination of superoxide using pyrogallol red

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A simple and fast spectrophotometric methodology able to quantify superoxide released by NADPH oxidase from differentiated promyelocytic leukaemia (HL-60) cells using pyrogallol red is described.The latter is based on the known stoichiometry of the reaction between superoxide and pyrogallol red and the inability of pyrogallol red to react with hydrogen peroxide. In addition, we developed a 96-wells microplate-based method able to determine NADPH oxidase activity. Using this method, we determined pharmacological properties of the NADPH oxidase inhibitors VAS2870 and diphenyleneiodonium and the obtained IC50 values were in good agreement with previous reported data. NOX2 is highly expressed in differentiated promyelocytic leukaemia cells, whereas other isoforms are not detected or expressed at low amounts. Likewise, this methodology may be a useful assay for NOX2 inhibitor screening. NADPH oxidases are involved in several physiological and pathological processes, rend