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TURN-ON CHEMILUMINESCENCE BIOASSAY FOR RAPID AND SENSITIVE QUANTIFICATION OF INTRECELLULAR H_2O_2 AND FOR ANTIOXIDANT SCREENING IN HUMAN LIVING CELL

D. Calabria[1], M. Guardigli[1], M. Mirasoli[1], P. Simoni[2], M. Zangheri[1], E. Pagnotta[3], C. Caliceti[1], A. Roda[1]

[1]*Dipartimento di Chimica "G. Ciamician", Alma Mater Studiorum, Università di Bologna, Bologna, Italy*
[2]*Dipartimento di Scienze Mediche e Chirurgiche, Alma Mater Studiorum, Università di Bologna, Bologna, Italy*
[3]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria- CREA, Bologna, Italy*

A new rapid (less than 1 hour) and simple effect-based bioassay for the selective measurement of intracellular H_2O_2 in live cells is reported. The bioassay relies on an adamantylidene - 1,2 - dioxetane probe containing an arylboronate moiety that in the presence of H_2O_2 is converted to the correspondent phenol. This triggers the chemiluminescent decomposition of the probe, which emits green light with high efficiency[1]. Under optimized conditions LOD and LOQ of 0.15 µM and 0.50 µM H_2O_2, respectively, have been obtained (corresponding to 3×10^{-11} and 1×10^{-10} moles of H_2O_2). Taking advantage of its high selectivity and low detection limit, the probe has been successfully employed for the quantification of intracellular H_2O_2 in living human endothelial, colon and keratinocyte cells exposed to different pro-oxidant stimuli (i.e., menadione, PMA and LPS). Imaging of living cells[2] clearly reveals the chemiluminescence emission from cells after pro-oxidant stimuli. Treatment of cells with antioxidant molecules leads to a dose-dependent decrease of intracellular H_2O_2 during biological processes. As a proof of concept, the bioassay has been used to measure the antioxidant activity of extracts from a *Brassica juncea* (oriental mustard) "Broad-leaf" selection, containing glucosinolates, isothiocyanates and other antioxidant molecules.

(A) (B)

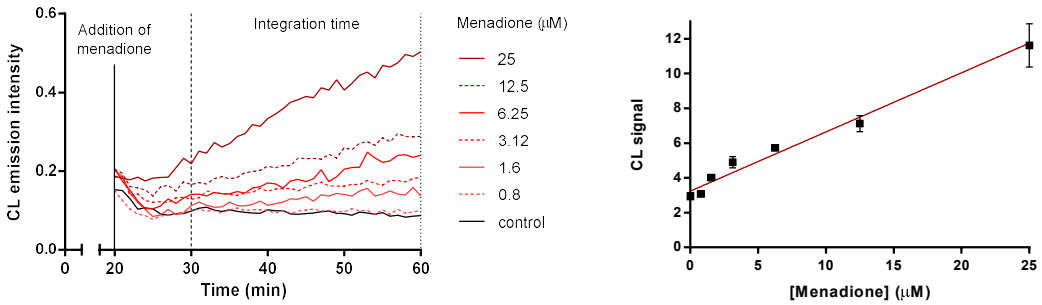

Figure 1. (A) Chemiluminescence kinetic profiles obtained for Caco-2 cells in the presence of the H_2O_2 CL probe and different concentrations of menadione. (B) Dose-response showing the correlation between the CL signal

and the concentration of menadione. Each point represents the mean ± SD of three independent measurements.

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Reference

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