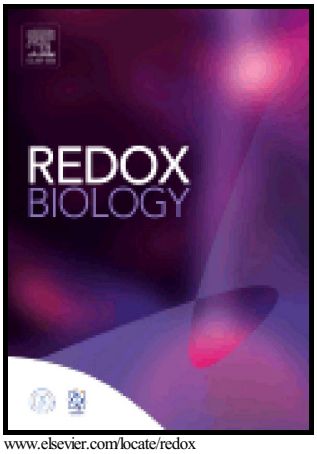

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PII: S2213-2317(18)30300-8
DOI: https://doi.org/10.1016/j.redox.2018.04.017
Reference: REDOX912

To appear in: *Redox Biology*

Received date: 13 April 2018
Accepted date: 17 April 2018

Cite this article as: Christos D. Georgiou, Dimitrios Zisimopoulos, Vasiliki Argyropoulou, Electra Kalaitzopoulou, Panayiotis V. Ioannou, George Salachas and Tilman Grune, Protein carbonyl determination by a rhodamine B hydrazide-based fluorometric assay, *Redox Biology,* https://doi.org/10.1016/j.redox.2018.04.017

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Abstract

A new fluorometric assay is presented for the ultrasensitive quantification of total protein carbonyls, and is based on their specific reaction with rhodamine B hydrazide (RBH), and the production of a protein carbonyl-RBH hydrazone the fluorescence of which (at ex/em 560/585 nm) is greatly enhanced by guanidine-HCl. Compared to the fluorescein-5-thiosemicarbazide (FTC)-based fluorometric assay, the RBH assay uses a 24-fold shorter reaction incubation time (1 hr) and at least 1000-fold lower protein quantity (2.5 µg), and produces very reliable data that were verified by extensive standardization experiments. The protein carbonyl group detection sensitivity limit of the RBH assay, based on its standard curve, can be as low as 0.6 pmol, and even lower. Counting the very low protein limit of the RBH assay, its cumulative and functional sensitivity is 8,500- and 800-fold higher than the corresponding ones for the FTC assay. Neither heme proteins hemoglobin and cytochrome *c* nor DNA interfere with the RBH assay.