

Dihydrorhodamine assay

(to measure peroxynitrite scavenging effects of antioxidant compounds)

Peroxynitrite scavenging effect was measured by monitoring the oxidation of DHR123 (Molecular Probes, Eugene Oregon) according to the method of (Kooy et al., 1994). DHR123 (5 μ M) was diluted in 90mM NaCl, 50 mM Na₂PO₄ pH: 7.4, 5 mM KCl and was measured into black 96 well plates (100 μ l/well). Oxidation of DHR123 by peroxynitrite (final concentration: 50 μ M) was measured with a microplate fluorescence spectrophotometer with excitation and emission wavelengths of 485/527, respectively at room temperature in the presence or absence of the test-compounds.

Ref.

Kooy NW, Royall J A, Ischiropoulos H and Beckman J S (1994) Peroxynitrite-Mediated Oxidation of Dihydrorhodamine 123. *Free Radic Biol Med* **16**:149-156.