CREATIVE BIOMART®
Assay Kit

Myeloperoxidase Inhibitor Screening Assay Kit

Product Information

Cat.No.

Kit-1982

Size

2 x 96 wells

Description

Myeloperoxidase (MPO) is a member of the heme peroxidase superfamily and is stored within the azurophilic granules of leukocytes. MPO is found within circulating neutrophils, monocytes, and some tissue macrophages. A unique activity of MPO is its ability to use chloride as a cosubstrate with hydrogen peroxide to generate chlorinating oxidants such as hypochlorous acid, a potent antimicrobial agent. Recently, evidence has emerged that MPO-derived oxidants contribute to tissue damage and the initiation and propagation of acute and chronic vascular inflammatory diseases. The fact that circulating levels of MPO have been shown to predict risks for major adverse cardiac events and that levels of MPO-derived chlorinated compounds are specific biomarkers for disease progression, has attracted considerable interest in the development of therapeutically useful MPO inhibitors. MPO also oxidizes a variety of substrates, including phenols and anilines, via the classic peroxidation cycle. The relative concentrations of chloride and the reducing substrate determine whether MPO uses hydrogen peroxide for chlorination or peroxidation. Assays based on measurement of chlorination activity are more specific for MPO than those based on peroxidase substrates because peroxidases generally do not produce hypochlorous acid. However, it is important that when screening for MPO inhibition that both the chlorination and peroxidation activities be tested. This determines whether the inhibitor specifically interferes with the chlorination and/or peroxidation cycle or whether the inhibitor simply acts as a scavenger for hypochlorous acid. Also, many reversible inhibitors act by diverting MPO from the chlorinating cycle to the peroxidase cycle. TheMPO Inhibitor Screening Assay provides convenient fluorescence-based methods for screening inhibitors to both the chlorination and peroxidation activities of MPO. The chlorination assay utilizes the non-fluorescent 2-[6-(4-aminophenoxy)-3-oxo-3H-xanthen-9-yl]-benzoic acid (APF), which is selectively cleaved by hypochlorite (-OCl) to yield the highly fluorescent

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compound fluorescein. Fluorescein fluorescence is analyzed with an excitation wavelength of 480-490 nm and an emission wavelength of 515-520 nm. The peroxidation assay utilizes the peroxidase component of MPO. The reaction between hydrogen peroxide and ADHP (10-acetyl-3,7-dihydroxyphenoxazine) produces the highly fluorescent compound resorufin. Resorufin fluorescence is analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Storage

4°C

Kit Components

MPO Assay Buffer: 1 vial; 4°CMPO Chlorination Substrate: 1 vial; 4°CMPO Peroxidation Substrate: 2 vials; 4°C Myeloperoxidase Control: 1 vial; -20°C MPO Inhibitor: 1 vial; 4°CMPO Hydrogen Peroxide: 1 vial; 4°CDMSO Assay Reagent: 1 ml; Room temperature96-Well Solid Plate (black): 2 plates; Room temperature96-Well Cover Sheets: 2 covers; Room temperature

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