CREATIVE BIOMART®
Assay Kit

Myeloperoxidase Chlorination Fluorometric Assay Kit

Product Information

Cat.No. Kit-0606

Product Overview

Myeloperoxidase Chlorination Fluorometric Assay provides a convenient fluorescence-based method for detecting the MPO chlorination activity in both crude cell lysates and purified enzyme preparations. The 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 compound fluorescein. Fluorescein fluorescence is analyzed with an excitation wavelength of 480-495 nm and an emission wavelength of 515-525 nm. The kit includes a MPO-specific inhibitor for distinguishing MPO activity from MPO-independent fluorescence.

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 (H2O2) 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 H2O2 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. The only exception is eosinophil peroxidase that produces hypochlorous acid at pH below 5. The chlorination activity of MPO has a neutral pH optimum, therefore the assay conditions can be set so that only MPO activity is specifically measured.

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Usage

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

Storage

Stability: 6 months;Storage: 4°C;Remove the Myeloperoxidase Control from the kit and store at -20°C. The rest of the components should be stored at 4°C. This kit will perform as specified if used before the expiration date indicated on the outside of the box.

Kit Components

For best results, remove components and store as stated below.MPO Assay Buffer: 1 bottle, 4°C;Fluorescein Standard 1: vial, 4°C;MPO Inhibitor 1: vial, 4°C;MPO Hydrogen Peroxide 1: vial, 4°C;Myeloperoxidase Control: 1 vial, -20°C;MPO Chlorination Substrate: 2 vials, 4°C;96-Well Solid Plate (black): 2 plates, Room temperature;96-Well Cover Sheet: 2 covers, Room temperature

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