Recombinant Human Myeloperoxidase/MPO

Catalog Number: 3174-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Myeloperoxidase/MPO protein Ala49-Ser745, with a C-terminal 10-His tag Accession # P05164
N-terminal Sequence Analysis	Ala49
Predicted Molecular Mass	80 kDa

SPECIFICATIONS

SDS-PAGE	74-106 kDa, under reducing conditions.
Activity	Measured by its ability to oxidize guaiacol in the presence of hydrogen peroxide. Capeillere-Blandin, C. (1998) Biochem J. **336** :395. The specific activity is >50,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM NaH_2PO_4, pH 7.0
- Recombinant Human Myeloperoxidase/MPO (rhMPO) (Catalog # 3174-MP)
- Hydrogen Peroxide Solution, 30% (v/v) (H_2O_2) (Sigma, Catalog # H1009)
- Guaiacol (Acros Organics, Catalog # AC120192500)
- Clear StripWell Microplate (Costar, Catalog # 92592)
- Plate Reader with Absorbance reading capability (Model: Spectramax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 100 mM guaiacol in Assay Buffer by shaking or stirring for 30 minutes at room temperature prior to use. Note: Protect guaiacol solution from light.
2. Dilute rhMPO to 1 µg/mL in Assay Buffer.
3. Dilute hydrogen peroxide from 30% to 0.00667% in Assay Buffer.
4. Load in a clear microplate 20 µL of 1 µg/mL of rhMPO and 30 µL 0.00667% hydrogen peroxide, and start the reaction by adding 50 µL of 100 mM guaiacol.
5. Read at 470 nm in kinetic mode for 5 minutes.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x Conversion Factor** (pmol/OD)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using known concentrations of hydrogen peroxide ranging from 20 to 300 µM. Each point contains 10 µg/mL of rhMPO (an amount so that the reaction will be completed in a short period of time) and 50 mM guaiacol. After each reaction is complete, the product is measured at 470 nm (read endpoint about every 20 seconds to find the maximum absorbance for each point). The maximum values of Abs470 (y-axis) and pmol of hydrogen peroxide (x axis) for each point is plotted linearly (y = mx + b) and the slope is calculated (m). The conversion factor is derived from the following equation as a unit of pmol/OD. It is multiplied by 2 because one mol of hydrogen peroxide is equal to two mol of oxidized guaicol (product).

Conversion Factor = (1/slope(m)) x 2 = pmol/OD

Final Assay Conditions

Per Well:

- rhMPO: 0.020 µg
- Hydrogen Peroxide: 0.002%
- Guaiacol: 50 mM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5-1 mg/mL in sterile, deionized water.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Myeloperoxidase (MPO) is a heme-containing enzyme belonging to the XPO subfamily of peroxidases. It is an abundant neutrophil and monocyte glycoprotein that catalyzes the hydrogen peroxide-dependent conversion of chloride, bromide, and iodide to multiple reactive species (1). Post-translational processing of MPO involves the insertion of a heme moiety and the proteolytic removal of both a propeptide and a 6 aa internal peptide (2). This results in a disulfide-linked dimer composed of a 60 kDa heavy and 12 kDa light chain that associate into a 150 kDa enzymatically active tetramer. The tetramer contains two heme groups and one disulfide bond between the heavy chains (2). Alternate splicing generates two additional isoforms of MPO, one with a 32 aa insertion in the light chain, and another with a deletion of the signal sequence and part of the propeptide (3). Human and mouse MPO share 87% aa sequence identity. MPO activity results in protein nitrosylation and the formation of 3-chlorotyrosine and dityrosine crosslinks (4-6). Modification of ApoB100, as well as the lipid and cholesterol components of LDL and HDL, promotes the development of atherosclerosis (5, 7-9). MPO is also associated with a variety of other diseases (1), and inhibits vasodilation in inflammation by depleting the levels of NO (10). Serum albumin functions as a carrier protein during MPO movement to the basolateral side of epithelial cells (11). MPO is stored in neutrophil azurophilic granules. Upon cellular activation, it is deposited into pathogen-containing phagosomes (2). While mice lacking MPO are impaired in clearing select microbial infections, MPO deficiency in humans does not necessarily result in heightened susceptibility to infections (12, 13).

References:

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