Recombinant Mouse Myeloperoxidase/MPO

Catalog Number: 3667-MP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse Myeloperoxidase/MPO protein Met16-Thr718, with a C-terminal 10-His tag Accession # AAR99349
N-terminal Sequence Analysis	Met16
Predicted Molecular Mass	81 kDa

SPECIFICATIONS

SDS-PAGE	60-70 kDa and 85-95 kDa, 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 >8,000 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 PBS. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 20 mM MOPS, 0.1 M NaCl, 1 mM $CaCl_2$, pH 7.0
- Recombinant Mouse Myeloperoxidase/MPO (rmMPO) (Catalog # 3667-MP)
- Hydrogen Peroxide Solution, 30% (w/w) (H_2O_2) (Sigma, Catalog # H1009)
- Guaiacol (Acros Organics, Catalog # AC120192500)
- Quartz Cuvette (Starna Cells, Catalog # 9B-Q-10) or equivalent
- Spectrophotometer with cuvette port (Model: Spectramax Plus by Molecular Devices) or equivalent

Assay

1. Prepare the substrate mixture by diluting guaiacol to 100 mM in Assay Buffer containing 0.0034% H_2O_2.
2. Shake or stir for 15 minutes at room temperature. Protect from light.
3. Dilute rmMPO to 3.34 µg/mL in Assay Buffer.
4. Load into a quartz cuvette 400 µL of 3.34 µg/mL rmMPO and start the reaction by adding 400 µL of the diluted guaiacol/H_2O_2 mixture. As a Substrate Blank combine 400 µL of Assay Buffer and 400 µL of the diluted guaiacol/H_2O_2 mixture (note: it is essential to monitor the reaction immediately after the introduction of the substrate mixture).
5. Read each cuvette at 470 nm in kinetic mode for 1 minute. Use only the first 10 seconds of data in the activity calculation.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Absorbance change per minute } (\Delta A/\text{min}) \times \text{sample volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff } (M^{-1}cm^{-1}) \times \text{amount of enzyme (µg)}}$$

Notes:
Absorbance readings are adjusted for the Substrate Blank
Use an extinction coefficient of 5580 $M^{-1}cm^{-1}$
The output of many spectrophotometers is in milli absorbance units per minute in kinetic mode

Final Assay Conditions

Per Reaction:

- rmMPO: 1.336 µg (20 nM)
- H_2O_2: 0.0017% (0.5 mM)
- Guaiacol: 50 mM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/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 human 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). Mouse and human 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).

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