Myeloperoxidase Assay Kit (500 assays)
Catalog Number: CB6937
Storage Temperature: 4 °C

Introduction

Myeloperoxidase (MPO) is the most abundant proinflammatory enzyme in neutrophilic granulocytes, accounting for approximately 5% of their dry mass. It catalyzes the formation of hypochlorous acid from hydrogen peroxide, generates other highly reactive molecules such as tyrosyl radicals, and cross-links proteins. MPO is implicated in a multitude of diseases, including atherosclerosis, myocardial infarction, atrial fibrillation, multiple sclerosis, Alzheimer's disease, lung cancer, and transplant rejection.

We have developed a simple, rapid and highly sensitive assay to measure cell and tissue MPO activity for wide analyses of multiple types of tissues, organs. The assay will be performed within two hours from sample preparation to data availability.

Kit Components (for 500 assays in 96 well plates)

A: Sample preparation buffer (100 ml) – Buffer A
B: Pre-reaction buffer (50 ml) – Buffer B
C: Stop buffer (50 ml) – Buffer C
D: H_2O_2 solution (500 µl) – Buffer D
E. Dianisidine dihydrochloride solution (500 ul) – Buffer E

Note: To make 1 ml of **Ready-to-use Reaction Buffer**, freshly add Solution D (10 ul) and Solution E (10 ul) into the Pre-reaction Butter B (980 ul).

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate or spectrophotometer cube
- Spectrophotometric multi-well plate reader or Beckman spectrophotometer
- Tissue glass homogenizer or grinder
- PBS
- Proteinase inhibitor

Procedure

All samples and standards should be run in triplicate.

1. Sample Preparation: Tissues were homogenized in PBS containing proteinase inhibitor and centrifuged at 8,000 × g for 5minutes at 4 °C. Remove supernatants, and the pellets were solved in sample preparation Buffer A (Freshly add protease inhibitor cocktails) and rotated at 4 °C cold room for 1 hour. Centrifuge at 13,000 × g for 10 minutes at 4 °C. The supernatants were collected for direct MPO assay. Sample protein concentration was determined by BCA method. Note: Serum samples may be assayed directly or diluted in MPO reaction Buffer
2. MPO reaction: 20 µl of the protein samples (about 20 µg) were added into 100 µl of freshly prepared **ready-to-use Reaction Buffer** (98 µl Buffer B + 1 µl Buffer D + 1 µl Buffer E, freshly prepared) for 1-5 min.
3. Reaction was stopped by adding 80 µl of Stop Buffer C for 5 minutes.
4. Absorption was measured at 460 nm to estimate MPO activity.
   Data are expressed as A460/Min/g sample protein.

Reference

Immunity. 2018 Jul 17;49(1):56-65. The TWIK2 Potassium Efflux Channel in Macrophages Mediates NLRP3 Inflammasome-Induced Inflammation. Di A1, Xiong S1, Ye Z1, et al.J Clin Invest. 2017 Nov 1;127(11):4124-4135. Caspase-11-mediated endothelial pyroptosis underlies endotoxemia-induced lung injury. Cheng KT, Xiong S, Ye Z, et al.