Pulmonary Myeloperoxidase Activity

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[Abstract] Neutrophils are considered one of the first responders of the innate immune response. Their primary activities are to migrate to sites of infection by chemotaxis and trans-migration across the endothelium (Gaines *et al.*, 2005). Once at the site of infection, they phagocytize microbes and kill them. Critical to the neutrophil's ability to kill microbes are the multiple degradative enzymes contained within granules. The activity of these enzymes is non-specific, and therefore, neutrophils also contribute to tissue damage at the site of infection (Gaines and Berliner, 2005). Measurement of neutrophil infiltration into tissues is one way to gauge the severity of infection, inflammation, and tissue damage (Ayala *et al*., 2002). Myeloperoxidase is found in the primary granules of neutrophils and is an effective measure of neutrophil infiltration into tissues (Gaines and Berliner, 2005).

Materials and Reagents

1. Fresh or snap-frozen tissues
2. Liquid nitrogen
3. MPO Fluoremetric Detection Kit (Assay Designs, catalog number: 907-029)
4. N-ethylmaleimide (Sigma-Aldrich, catalog number: E2068)
5. Hexadecyltrimethylammonium (Sigma-Aldrich, catalog number: 52366)
6. Deionized water
7. DMSO (Sigma-Aldrich, catalog number: D8418)
8. Assay buffer (see Recipes)
9. Hydrogen Peroxide (see Recipes)
10. Detection Reagent (see Recipes)
11. Reaction cocktail (see Recipes)

Equipment

1. 14 ml Polystyrene test tubes
2. 2 ml Microfuge tubes

3. Black-welled 96 well plates
4. Polytron homogenizer
5. Sonicator
6. Fluorescent plate reader
7. Refrigerated centrifuge

Procedure

1. Weigh out 50 mg tissue into polycarbonate test tubes containing 0.5-1 ml ice cold 1x assay buffer with 10 mM N-ethylmaleimide.
2. Homogenize to just disrupt the tissue by placing the tip of the homogenizer in the bottom of the tube and switching the machine on, agitating the tube to move the tip of the homogenizer throughout the liquid and then switching the machine off after approximately one sec. Repeat until the tissues are just disrupted, usually 9 more times for a total of 10 pulses.
3. Centrifuge at 500 *x g* for 10 min at 4 °C.
4. Discard supernatant, add 500 µl ice cold 1x assay buffer containing 0.5% hexadecyltrimethylammonium to the pellet and transfer to a 2 ml microfuge tube.
5. Homogenize to lyse the cells by placing the tip of the homogenizer in the bottom of the tube and switching the machine on, agitating the tube to move the tip of the homogenizer throughout the liquid and then switching the machine off after approximately 5 sec. Repeat 9 times for a total of 10 pulses. Place on ice.
6. Sonicate to further lyse the cells at 50% power for 10 sec. Place on ice. Repeat 2 times for a total of 3 pulses.
7. Snap freeze in liquid nitrogen and thaw at room temperature by placing in liquid nitrogen, immediately removing from the nitrogen and leave the samples at room temperature until completely thawed. Repeat snap freeze and thaw once.
8. Store at -80 °C until assayed.
9. Prior to assay, serially dilute the included MPO standard in assay buffer.
10. To assay, add 50 µl of sample or the MPO standard to the bottom of a black 96 well plate. Add 50 µl Reaction Cocktail provided by the kit. All samples and standards should be run in duplicate.
11. Incubate at room temperature in the dark for 30 min.
12. Read the fluorescence at 550 nm excitation and 595 nm emission every 10 min until 60 min incubation.
13. Plot the standard concentration vs. the maximum relative fluorescence units to create a standard curve (Figure 1).

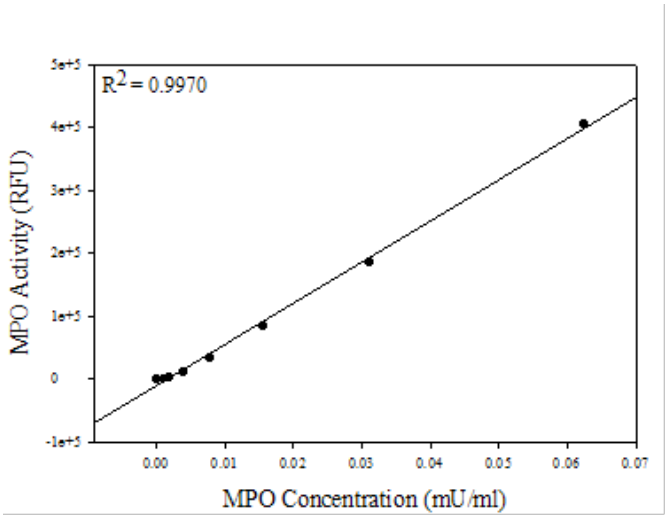

Figure 1. Representative Standard Curve

14. Determine the concentration of the unknowns from the standard curve.

Recipes

1. 1x Assay buffer
   4 ml MPO assay buffer concentrate
   36 ml deionized water
2. Hydrogen Peroxide
   22.7 µl 3% hydrogen peroxide
   977 µl deionized water
3. Detection Reagent
   Supplied vial
   500 µl DMSO
4. Reaction cocktail
   50 µl detection reagent
   5 µl hydrogen peroxide
   4.875 ml 1x assay buffer

Acknowledgments

This protocol is adapted from Ozment *et al.* (2012).

References

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