Northwest
Life Science Specialties, LLC

Premier Products for Superior Life Science Research

NWLSS™ Superoxide Oxidant Status Assay

Product NWK-SOS01

For Research Use Only

Assay kit for quantitative measurement of superoxide in monocyte cell populations and /or oxidant activity in other biological samples.

Table of Contents

Introduction:

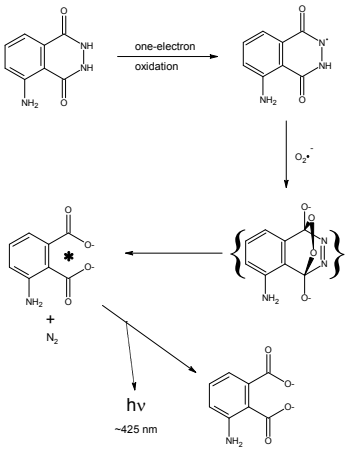
Superoxide and other reactive oxygen species play very important role in diseases and pathology related to oxidative stress. Their extremely low steady-state concentration *in-vivo* require sensitive methods of measurement. A number of techniques have been described in the literature including *ferricytochrome c* reduction, nitroblue tetrazolium reduction, aconitase activity inhibition, nitrone spin trapping, electrochemical detection, and chemiluminescent assays with luminol and lucigenin. The luminol based assay has been criticized as a specific quantitative method to determine superoxide production in cells due to its sensitivity to a number of reactive oxygen species (hypochlorite, peroxynitrite and hydroxyl radical from H_2O_2+metal/heme-proteins). Also, the first intermediate in the reaction can react with oxygen to generate superoxide resulting in higher apparent superoxide production rate than actual cellular production rate. However, this assay can provide a valid, simple and sensitive gross assessment of reactive oxygen species in many biological sample types and is an important method in the analysis of specific superoxide production by monocytes.

Intended Use:

The NWLSS™ Superoxide Oxidant Status Assay provides a chemiluminescent method for determination of overall oxidant levels in biological samples and is largely specific for superoxide levels when testing isolated populations monocyte cells.

Test Principle:

The NWLSS™ NWK-SOS01 method is based on Luminol reaction with superoxide to produce a luminophore with an emission peak at ~425 nm (scheme 1). The luminescence intensity is proportional to the amount of superoxide in the sample.



A number of compounds such as iodophenol are known to increase the intensity of luminescent emission from luminol and are widely used in immunological protein and DNA detection kits. However, their toxicity limits the usage only in cell-free systems. In contrast, orthovanadate can increase chemiluminescence by approximately 50X, is not toxic to cells and does not induce chemiluminescence by itself. For these reasons, the NWLSS™ Superoxide Oxidant Status Assay kit utilizes sodium orthovanadate to increase the sensitivity to superoxide.

The mechanism of orthovanadate action is not completely understood; it is believed that orthovanadate may act as an electron transporter or may lower activation energy for luminol activation. In any case, the kit's sensitivity to different types of oxidants (and therefore its specificity) should be considered different if used with and without enhancer.

For monocytic cells, the enhanced signal after stimulation is inhibited by superoxide dismutase (SOD), but not significantly by catalase (an H_2O_2 scanvenger) and not by azide (a myeloperoxidase inhibitor).

Inhibitor	Relative Chemiluminescence
None (control)	3948 +/- 27
Superoxide Dismutase	87 +/- 1
Catalase	3051 +/- 68
Azide	3095 +/- 70

The signal is also linearly proportional to the number of monocyte cells up to approximately 10^6 cells. This further indicates that superoxide is mainly responsible for the chemiluminescent signal in these cells. Caution should be taken in extending these results to other cell types and model systems where a different oxidant may be the more dominant species produced.

General Specifications:

Format:	Cuvette or 96 Well Microplate
Number of Tests:	30 Cuvette or 96 Microplate
Specificity:	Gross Oxidant Status in Biological Samples
	Superoxide in Monocyte Cell Populations
Sensitivity:	LLD = 0.3 U/mL in Reaction Mix
	5 U/mL in Sample Added to Reaction Mix

Kit Contents:

Luminol Solution	1 X 3 mL
HBSS Buffer Solution (endotoxin free)	1 X 30 mL
Enhancer	1 Vial
Phorbol Myristate Acetate (PMA)	1 Vial (0.025 mg)
DMSO (PMA Solvent)	1.1 mL

Required Materials Not Provided:
Disposable, glass or polystyrene tubes (12x75 mm or other sizes suitable for luminometers) or white (black) polystyrene plates suitable for luminescence plate readers).
Millipore or double distilled water (resistance ^{3} 18 MW/cm @ 25°C).
Pipetters, adjustable 0.0-1.0 mL.
Disposable pipette tips.
Pipetter, 10.0 mL.
No material is provided for tissue cell cultures and other sample preparation reagents.

Required Instrumentation:
Luminometer (single tube or plate reader).

Warnings, Limitations, Precautions:
Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Note: PMA is a potent tumor promoter, use extreme caution when handling this compound. Avoid any contact with skin.

Storage Instructions:
Upon receipt, store the reagents at 2-8°C. Do not use components beyond the expiration date printed on the label.

Unopened solutions are stable for 9 months if stored at 2-8°C. . Dispose solutions after kit's expiration date.

After reconstituting with solvents, the enhancer and PMA solutions should be stored at –20°C. when not in use.

Assay/Instrument Preparation:
Luminometer Setup
Set luminometer or plate reader temperature at 25.0°C. or 37.0°C. , otherwise use room temperature if it is stable throughout the experiment.

Reagent Preparation:
Allow the kit to wcome to room temperature completely before use.

Enhancer:
Pipet 1.0 mL of dH_2O to the enhancer vial provided. Label as *Working Enhancer*. Store this solution at –20°C again immediately after each use. *Working Enhancer* reagent can undergo multiple freeze/thaw cycles.

PMA (If used):
Pipet 1.0 mL DMSO from DMSO Bottle into the PMA vial. Dissolve PMA by shaking for half minute. Re-label the Bottle as a PMA/DMSO Reagent. Store this solution at –20ºC immediately after experiment. The PMA/ DMSO Reagent can undergo multiple freeze/thaw cycles.

Note: Always use extreme precaution when handling the PMA compound and reconstituted reagent. Wear gloves to avoid skin contact.

Sample Dilution Buffer:
Supplied Ready to Use.

Sample Handling/Preparation:
Cells
Typically >10^5 cells are needed per assay. Samples may need to be diluted or concentrated (by centrifuging and re-suspending).

Whole Blood
2.5 uL (400X dilution in reaction mix) is typically enough whole blood to produce an easily measurable reaction if stimulant is used however it is up the researcher to determine the optimal sample volume required for non-stimulated samples which will depend heavily on the activation state of circulating leucocytes.

Tissue Homogenates:
It is up the researcher to determine the optimal sample volume required for tissue homogenate samples and this will depend heavily on the type, number and activation state of infiltrating cells.

Luminometer Tube Assay Protocol:
Note: The targeted concentrations of Luminol and PMA in the assay medium are 60 μM and 500 μg/L, respectively. When higher sample volumes are required to achieve increased sensitivity, users should increase volumes of these reagents as well.

1. Set luminometer (plate reader) temperature at 25.0ºC or 37.0ºC , else use room temperature if it is stable throughout the experiment.

2. Determine the volume of samples (Vs) needed to run the assay.

3. To a glass or polystyrene tube, pipet (1000 – 100 - V_s – V_i) μL of HBSS Buffer or sample re-suspended in HBSS buffer where Vi is the volume of inhibitor added if one is used. If no stimulant is to be used pipet (1000 - 80 - V_s - Vi).

4. Pipet 60 μL of the Luminol Solution.

Assay Protocol (continued)

5. Pipet 20 µL of the Enhancer Solution.

6. Add V_i µL of Inhibitor Solution (if used).

7. Add V_s µL of samples, mix well.

8. Add 20 µL of PMA (if used) or other stimulator solutions, mix well.

9. Place the tube into the luminometer immediately.

10. Record the luminescence at desired time interval (i.e. every 5 seconds) for 30 minutes or until reaching a luminescence plateau).

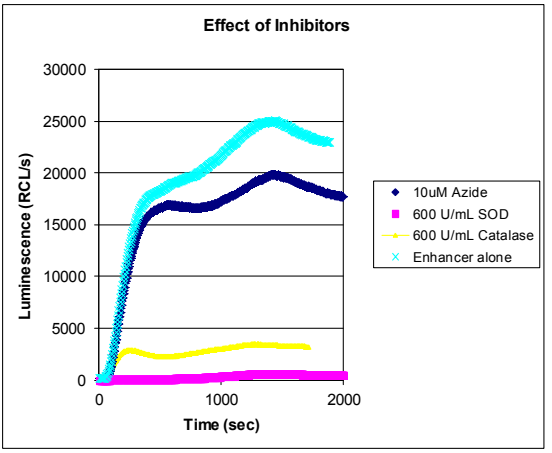
11. The superoxide/TOS production rate is expressed as relative chemiluminescene intensity (RCL/s). The plateau intensity represents the maximal superoxide/TOS of the sample being tested.

Luminescence Capable Plate Reader Assay Protocol:

The reagent volumes recommended in the previous procedure are designed for measurement with a single-tube luminometer. For assays with a micro plate luminescence reader, 1/3x volumes should be used in each step.

Example Assay Performance:

The figure below shows luminescence production as a function of time when a whole blood sample (2.5 µL in a 1.0 mL total assay volume) was stimulated with PMA, in the presence of the enhancer and inhibitors at 25.0°C. The luminescence intensity was only ~1/20x in the absence of the enhancer and little luminescence was observed without PMA. A single-tube luminometer was used to record the data.



Performance Details:
Intra-assay precision for a 2.5 uL whole blood sample was calculated as 9% CV (n=3). The high CV is mainly a reflection of the precision that can be achieved in pipetting 2.5 μL of blood samples.

References:
1. Pfefferkorn, United States Patent # 5,492,816 (1996).

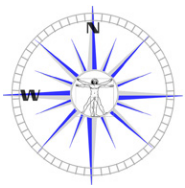
2. Allen R. C., "Phagocytic Leukocyte Oxygenation Activities and Chemiluminescence: A Kinetic Approach to Analysis" *Method in Enzymology* Vol 133, 449-493 (1986).

Statement of Limited Warranty:
Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Notes:

Notes:

Notes:



Northwest
Life Science Specialties, LLC

5131 NE 94th Avenue, Suite 201
Vancouver, WA 98662
Phone 360-449-3091 or Toll Free: 888-449-3091
Fax 360-449-3092
E-mail: nwsales@nwlifescience.com