ASTARTE™ Biologics

Nitric Oxide Assay Kit

Catalog #K-8002

INTRODUCTION

Nitric oxide is a reactive nitrogen compound that acts as a signaling molecule as well as having antimicrobial potential. Endothelial cells express a constitutive nitric oxide synthetase, but inflammatory macrophages and other myeloid cells can be induced to express nitric oxide synthetase which converts arginine to citrulline and nitric oxide.

PRINCIPLE OF THE ASSAY

Nitric oxide is rapidly oxidized further to nitrite (NO_2) and often further oxidized to nitrate (NO_3). This kit uses the Greiss reaction to detect nitrites. Sulfanilic acid reacts with nitrite to form a diazonium salt which then reacts with naphthylethylenediamine to form a colored end product. This azo dye has its maximum absorbance between 540 and 570 nm, and absorbance is proportional to the concentration of nitrite.

REAGENTS INCLUDED

- Greiss Reagent, 100 mL
- 1 mM Nitrite Standard, 30 mL

REQUIRED EQUIPMENT

- Microplate Spectrophotometer
- Pipettes
- Clear Microtiter Plate (96 Well Format)

PRECAUTIONS

- Greiss reagent contains acid and can irritate skin or eyes. Wear appropriate protective equipment (lab coat, gloves and eye protection).

SAMPLE PREPARATION

Samples should be cleared of any cells or debris by centrifugation prior to use in the assay.

ASSAY PROCEDURE

Prepare a standard curve using the supplied sodium nitrite. If the samples to be tested are in tissue culture medium, then the standards should be diluted in the same tissue culture medium. A suggested format is shown in the table below.

Volume of 1 mM Sodium Nitrite	Volume of Water or Culture Medium	Final Concentration
0	1 mL	0
1 µL	999 µL	1 µM
3 µL	997 µL	3 µM
10 µL	990 µL	10 µM
30 µL	970 µL	30 µM
50 µL	950 µL	50 µM

Add 100 µL of sample or standard per well of a 96-well microtiter plate. Add an equal volume of Greiss reagent to each sample. Wait 15 minutes at ambient temperature, then read absorbance at 540–570 nm.

Samples and standards should be assayed in duplicates.

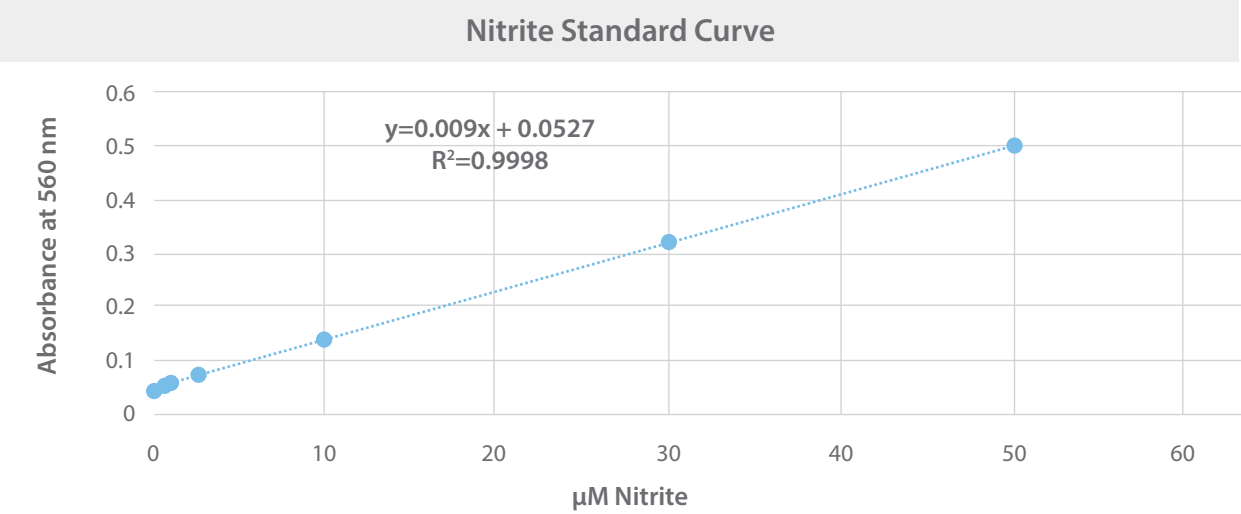
CALCULATION OF RESULTS

Plot the standard curve of absorbance (y-axis) versus nitrite concentration (x-axis) and calculate linear regression (y=mx+b where m is the slope and b is the y-intercept).

Calculate sample concentrations from the sample absorbance by solving for x [x=(y-b)/m].

EXPECTED RESULTS

The standard curve should be a straight line with a correlation coefficient close to 1 as shown in the graph below.



Mouse dendritic cells will produce NO after stimulation with LPS as shown in the graph below. For this experiment, mouse bone marrow derived DC (lot no. 3711OC17) were thawed and cultured in a 96-well plate at 50,000 cells per well. LPS was added at 100 ng/mL and culture medium was collected after 24, 48 and 72 hours. NO, as measured by nitrite concentration, was produced at 24 and 48 hours and began to level off at 72 hours.