GRIESS ASSAY FOR NITRITE DETERMINATION

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BACKGROUND

- Colorimetric assay method for measurement of total nitrites concentration in supernatant, with a detection limit of 1.5 µM
- Nitrite is inert oxidized product of nitric oxide, it is also the physiological storage pool of nitric oxide

NOTES

- Griess Reagent Kit for nitrite determination purchased from life technologies. Catalog number: G-7921 http://www.lifetechnologies.com/order/catalog/product/G7921
- The Kit affords the analyses of ca. 2500 individual samples
- Protocol modified from Griess Reagent Kit for Nitrite Determinate (G-7921) manuals & protocol provided by life technologies.
- For better results, use phenol red free growth medium for cell culturing

EQUIPMENT

- 96 well flat bottom plate (Felcon)
- Deionized water
- 1 M H_2SO4
- Griess Reagent Kit from life technologies
    - Reagent A
    - Reagent B
    - Nitrite standard solution (1.0 mM)

PROCEDURE

1. Prepare 90 mL 1.0 M H2SO4 from concentration H_2SO_4 (96%, 18M)
    a. Pour 85 mL deionize water in autoclaved 100 mL bottle
    b. Add 5 mL concentrated H_2SO_4, mix gently

2. Prepare 1 mL 1.0 mM nitrite standard solution (FW=69)
    a. Prepare 100 mM nitrite solution: 0.0069 g of sodium nitrite in 1 mL deionized water
    b. Prepare 1 mM nitrite solution: 10 µL of 100 mM nitrite solution in 990 µL deionized water

3. Prepare nitrite standards for one 96 well plate
   2x serial dilution from nitrite standard solution provided in the kit

Nitrite standard	Concentration (µM)	Nitrite solution (µL)	Deionize water (µL)	Final volume (µL)
1	100	60 µL from stock 1.0 mM solution	540	300
2	50	300 µL from solution 1	300	300
3	25	300 µL from solution 2	300	300
4	12.5	300 µL from solution 3	300	300
5	6.25	300 µL from solution 4	300	300
6	3.125	300 µL from solution 5	300	300
7	1.563	300 µL from solution 6	300	600
blank	0	0	600	600

4. Prepare Griess assay reagent by mixing the following:
   For one 96 well plate:
   1 mL reagent A
   1 mL reagent B
   8 mL deionized water
   5 mL 1.0 M H_2SO_4
5. In a 96 well microplate mix the following in each well
   a. 100-150 µL nitrite containing sample (cell supernatant/nitrite standard)
   b. 130 µL of Griess assay reagent mixture prepared in step 4.
6. Incubate the mixture for 30 min at room temperature
7. Measure the absorbance at 548 nm wavelength. Other wavelengths in the range of 520-590 nm can also be used if 548 nm was not available.
8. Convert absorbance reading to nitrite concentration based on calibration curve

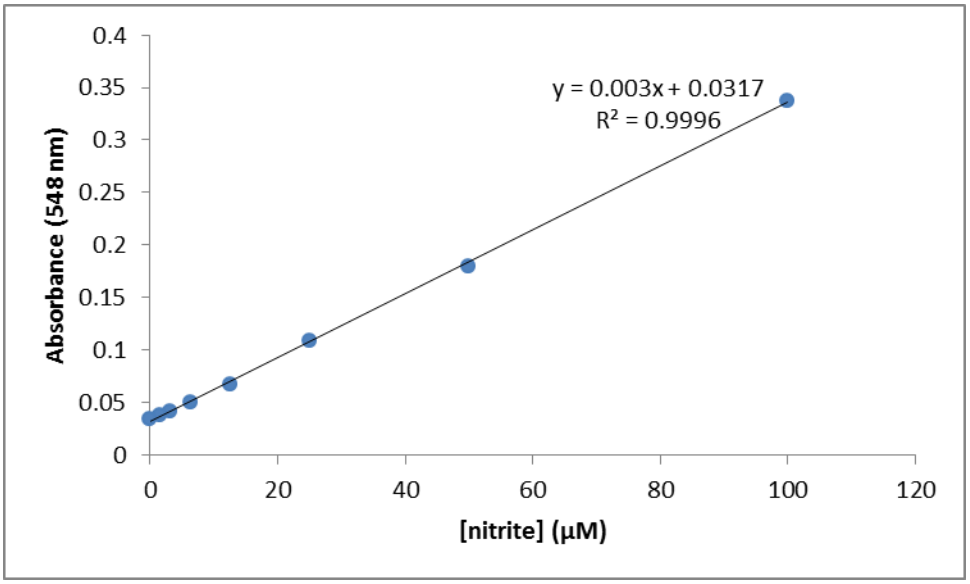
CAUTIONS

- Nitrate can be reduced to nitrite using nitrate reductase prior to the Griess assay.
- The reaction only occurs when the pH of the mixture (Griess reagent mixture and sample) dropped below 2. This can be achieved with the addition of 1.0 M H_2SO_4.
- Prepare enough Griess reagent mixture (step 4) for immediate use. Do not store mixture for more than 8 hrs.

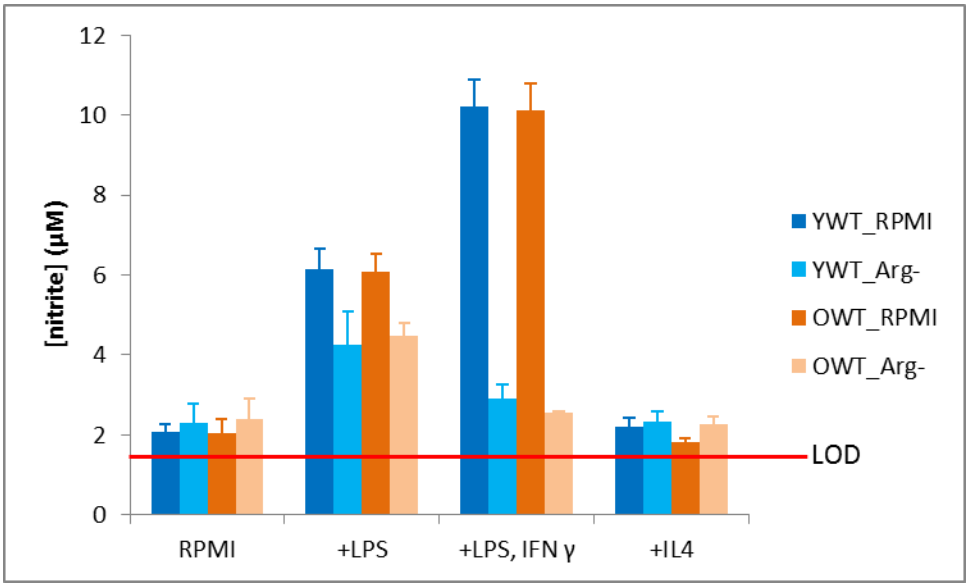
Example of 96well plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1										
B	Std 2	Std 2										
C	Std 3	Std 3										
D	Std 4	Std 4			Samples							
E	Std 5	Std 5										
F	Std 6	Std 6										
G	Std 7	Std 7										
H	blank	blank										

Calibration solution for sodium nitrite:



Example data:



Bone marrow derived macrophages (BMDM)

YWT—BMDM from 6-8 week old C57BL/6 Charles River mice; OWT—BMDM from 24 month old C57BL/6 Charles River mice; LPS—100 ng/mL; IFN γ—20 ng/mL; IL-4—20 ng/mL.