

Griess test protocol

Written by: Duha Al-Awad

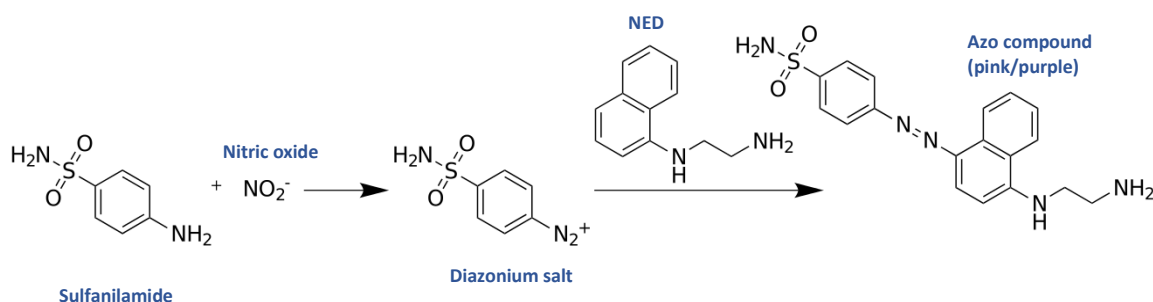
Date: 25th April 2019

Approved by: Dr. Susu Zughair

Protocol Number: P001

Background:

Nitric oxide is an important messenger molecule that is released during several different physiological & pathological processes. Nitric oxide is unstable and breaks down into stable nitrates and nitrites few seconds after its release. The Griess test (named after Peter Griess) detects the presence of nitrite ions (NaNO_2) in a solution, therefore, it is an indirect measurement of nitric oxide release. The Griess reaction is a diazotization reaction, where a diazonium salt is formed from the reaction between sulfanilamide and nitrite present in the sample. The diazonium salt is then complexed with an azo dye to form a colorful azo compound (pink).



Reagents:

1% Orthophosphoric acid

Sulfanilamide

Sodium nitrite

N-(1-Naphthyl)ethylene diamine dihydrochloride ACS (NED)

Materials:

96-well plate

Multichannel pipette

200 μL pipettes

Aluminum foil

50mL conical tube

Reagent preparation:

Reagent A: 1g sulfanilamide in 100 mL of 1% orthophosphoric acid (to make Sulphanilic acid)

Reagent B: N-(1-Naphthyl)ethylene diamine dihydrochloride: 0.1g NED in 100mL distilled H_2O

Working solution: mix equal volumes of reagent **A** & **B** in a dark bottle. Store at 4°C for later use. *It is preferable to mix reagents **A** & **B** right before use.

Standards Preparation:

1. To prepare a stock of **1M** NaNO₂ standard measure 0.69g of NaNO₂ to 10mL distilled water and vortex until the NaNO₃ dissolves completely in the water.
2. From the 1M stock, dilute the solution to 100 μM in two steps:
 - a. Add 10 μL (of 1M NaNO₂ stock) in 990 μL of distilled water to get a concentration of **10 mM** (0.01M).
 - b. Add 10 μL (from the 10 mM intermediate solution) in 990 distilled water to get a final concentration of **100 μM** (0.10mM).

Procedure:

1. Allow reagents to come to room temperature (15-30 mints incubation at RT).
2. Add 100 uL of each standard.
3. Add 100 uL of sample for neat runs. Add 50 uL of media and 50 uL of sample to dilute the samples by a factor of 1/2. *Multiply the result of the diluted samples by the dilution factor '2'.
4. Add 100 uL of the working solution to each well.
5. Blank contains media & Griess reagent only.
6. Read the absorbance at **540nm** within 30 mints
7. Construct a standard curve and find the unknown values.






*Nitrates (NO₃) can be enzymatically converted to nitrites (NO₂), however, the benefit is minimal, and hence it is not performed.

Storage Conditions:

Reagent	Storage
1% Orthophosphoric acid (solution)	Room temperature
Sulfanilamide (powder)	Room temperature
Sodium nitrite (powder)	Room temperature
N-(1-Naphthyl)ethylene diamine dihydrochloride ACS (NED) (powder)	Room temperature & protected from light
Sulfanilamide in orthophosphoric acid (soluble)	2-4 °C
N-(1-Naphthyl)ethylene diamine dihydrochloride ACS (NED) (soluble)	2-4 °C & protected from light

Hazards:

Reagent	Hazard
Sodium nitrite	None

Sulfanilamide	 May cause fire or explosion; strong oxidizer.  Toxic if swallowed.  Very toxic to aquatic life.
N-(1-Naphthyl)ethylene diamine dihydrochloride ACS (NED)	 <ul style="list-style-type: none"> - Acute toxicity (oral, dermal, inhalation), category 4 - Skin irritation, category 2 - Eye irritation, category 2 - Skin sensitization, category 1 - Specific Target Organ Toxicity Single exposure, category 3
Orthophosphoric acid	 Concentrated solution causes severe skin burns and eye damage.