BioVision

Gentaur

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For research use only

Nitric Oxide Fluorometric Assay Kit

(Catalog #K252-200; 200 assays; Store kit at -20°C)

I. Introduction:

Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune response and apoptosis. Since NO is rapidly converted to nitrite (NO_2^-) and nitrate (NO_3^-), the total concentration of nitrite and nitrate is used as a quantitative measure of NO production. **BioVision's Nitric Oxide Fluorometric Assay Kit** provides an accurate and convenient measurement of total nitrate/nitrite concentration in a simple two-step process. In the first step, nitrate is converted to nitrite by nitrate reductase. In the second step, nitrite reacts with the fluorescent probe DAN (2, 3-diaminonaphthalene). NaOH enhances the fluorescent yield. The fluorescent intensity is proportional to the total nitric oxide production. The kit has been tested with culture media, plasma, and tissue homogenates.

II. Kit Contents:

Components	K252-200	Cap Code	Part Number
Assay Buffer	40 ml	NM	K252-200-1
Enzyme Cofactor	Lyophilized	Blue	K252-200-2
Enhancer	Lyophilized	Purple	K252-200-3
Nitrate Reductase	Lyophilized	Green	K252-200-4
Nitrate Standard	Lyophilized	Yellow	K252-200-5
Nitrite Standard	Lyophilized	Orange	K252-200-6
DAN Probe	1 ml	Amber Red	K252-200-7
Sodium Hydroxide	1 ml	Clear	K252-200-8
96-Well White Plate	2	------	K252-200-9
Plate Cover	2	------	K252-200-10

III. Reconstitution of Reagents:

1. **Assay Buffer:** The Assay Buffer is ready to use as supplied. Store at 4°C.
2. **Enzyme Cofactor:** Reconstitute with 110 µl of dH_2O to make 10 mM stock solution. Aliquot and store at -20°C. Freeze/thaw should be limited to 1 time. Dilute appropriate portion 10X to make 1 mM working solution. Keep on ice while in use. Working solution can be stored at 4°C for 6-8 hr.
3. **Enhancer:** Reconstitute with 1.2 ml of Assay Buffer. Keep on ice during use. Store at -20°C.
4. **Nitrate Reductase:** Reconstitute with 1.2 ml of Assay Buffer. Aliquot desired amount and store at -20°C. Keep on ice during use. Freeze/thaw should be limited to 1 time.
5. **Nitrate/Nitrite Standards:** Reconstitute with 1.0 ml of Assay Buffer, vortex to generate 10 mM standard each. Store at 4°C when not in use (**do not freeze!**). The reconstituted Standards are stable for 4 months when stored at 4°C.
6. **DAN Probe and Sodium Hydroxide:** Ready to use. Store at 4°C.

IV. Measurement of Nitrate + Nitrite:

1. **Preparation of Standards:** Add 5 µl of the reconstituted 10 mM nitrate/nitrite Standards to 995 µl Assay Buffer, vortex to generate 50 µM working Standard Solution. Add 0, 4, 8, 12, 16, 20 µl of the working Standard to 6 consecutive wells to generate 0, 200, 400, 600, 800, 1000 pmol/well standard. Bring the volume to 75 µl with Assay Buffer.

   Note: DAN Probe reacts with nitrite, not nitrate. For routine total nitrite/nitrate assay, you may prepare a Nitrate Standard Curve only. However, if you need to measure nitrite and nitrate concentrations separately, you may prepare a Nitrite Standard Curve in the absence of Nitrate Reductase in the Standard Curve and assay samples. Nitrate = Total – Nitrite.
2. **Prepare Samples:** Samples containing high protein concentration may need to be filtered through a 10 kDa MW cut-off filter (BioVision Cat # 1997-25) prior to assay. Add 0-75 µl of sample to the wells and adjust the volume to 75 µl with Assay Buffer.

   Notes: Typical urine nitrite and nitrate levels are in the 0.2 - 2 mM and 1 - 20 µM range respectively. Typical normal serum levels are 0 - 20 µM and 0 - 2 µM respectively with various disease states elevating these levels significantly. Plasma samples or tissue homogenates should be assayed with no more than 10 µl of undiluted sample. Phenol red and serum in cell culture media may decrease the reading, and thus a standard curve should be made in the same media.
3. Add 5 µl of the Enzyme Cofactor working solution to all wells.
4. Add 5 µl of the Nitrate Reductase to nitrate assay wells (unknowns and standards), add 5 µl of buffer in place of Nitrate Reductase (unknowns and standards) when you determine nitrite separately.
5. Cover the plate with the plate cover and incubate at room temperature (RT) for 1 - 4 hr. 1 hr = ~ 90 % conversion of Nitrate to Nitrite, 2 hr = ~ 95 % conversion, 4 hr = ~ 99 % conversion.
6. Add 5 µl of Enhancer to each well. Incubate 30 min to quench interfering compounds.
7. Add 5 µl of DAN Reagent to each well. Incubate for 10 min at RT.
8. Add 5 µl of NaOH to each well. Incubate for 10 min at RT.
9. Read the plate in a fluorometer using Ex = 360 nm and Em = 450 nm.

V. Calculations

1. **Plot Standard Curve:** Plot fluorescence vs. picomoles nitrate.
2. **Determine sample nitrate and nitrite concentrations:**

   C = [nitrate + nitrite] (µM) $= \left(\frac{fluorescence - y\ intercept}{slope} / sample\ volume\ (\mu l)\right) x\ dilution\ ^*$

   OR

   C = Sa/Sv, where Sa is the amount of Samples as read from Standard Curve (in pmol), and Sv is the volume of sample added to the well (in µl), multiplied by the dilution* factor.

   *Dilution is the sample dilution done prior to addition of the Sample to the well.

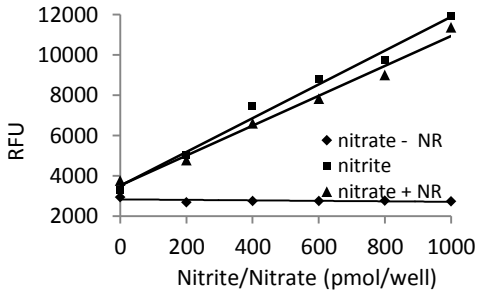

Figure: Nitrite, nitrate assay in the presence and absence of nitrate reductase. Assays were performed according to the kit protocol with 1 hr conversion of nitrate to nitrite at Step 5.

RELATED PRODUCTS:

- Nitric Oxide Colorimetric Assay Kit
- Glutathione Fluorometric & Colorimetric Detection Kits
- Lactate, Pyruvate, NADH/NAD, Glucose, Sucrose, Lactose, Maltose Assay Kits

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