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Nitrite Assay Kit (Griess Reagent)

rev 06/19

(Catalog # K544-200, -500, -1000; 200/500/1000 assays; Store at 4°C)

I. Introduction:

Nitrogen-based ions, nitrite and nitrate, are found in almost every living organism. Furthermore, endogenous Nitrite levels are found in mammals and also can be obtained from dietary sources. In humans, nitrite is further metabolized to Nitric Oxide and other reactive nitrogen species (nitrogen oxides). The Nitrate-Nitrite-NO biochemical pathway is well known for its participation in cell signaling, hypoxia-dependent response and regulation of blood flow. Recent studies suggest the reduction of Nitrite to Nitrogen Oxygen in the mitochondria. Specifically, myoglobin and xanthine oxidoreductase could generate NO under hypoxic conditions leading to mitochondrial respiration. BioVision's Nitrite Assay Kit utilizes the Griess Reagent, a classic protocol for the estimation of nitrite. In the assay, nitrite is reduced to Nitrogen Oxide using Griess Reagent I. Then, Nitrogen Oxide reacts with Griess Reagent II forming a stable product that can be detected by its absorbance at OD 540 nm. The one-step assay is simple, fast and can detect nitrite levels as low as 1 nmol/well.

Nitrite $\xrightarrow{\text{Griess Reagent I}}$ Intermediate $\xrightarrow{\text{Griess Reagent II}}$ Color detection (**OD: 540 nm**)

II. Applications:

- Detection of Nitric Oxide in various biological samples
- Analysis of cell signaling pathways (i.e. Nitrate-Nitrite-NO)

III. Sample Type:

- Animal tissues: liver, kidney, etc.
- Serum, plasma, urine
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K544-200	K544-500	K544-1000	Cap Code	Part Number
Nitrite Assay Buffer	30 ml	75 ml	150 ml	WM/NM/NM	K544-XX-1
Griess Reagent I	10 ml	25 ml	50 ml	NM	K544-XX-2
Griess Reagent II	10 ml	25 ml	50 ml	Amber	K544-XX-3
Nitrite Standard	1 vial	1 vial	1 vial	Orange	K544-XX-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Nitrite Assay Buffer, Griess Reagent I and Griess Reagent II:** Warm to room temperature (RT) before use. Store at 4°C.
- **Nitrite Standard:** Reconstitute with 100 µl Nitrite Assay Buffer to generate 100 mM Nitrite Standard solution. Store at 4°C. Keep on ice while in use. Reconstituted Standard is stable for 4 months when stored at 4°C.

VII. Nitrite Assay Protocol:

1. **Sample Preparation: Urine:** Dilute samples 10-fold using Nitrite Assay Buffer. Serum/Plasma. Deproteinize Samples using 10 kDa columns (Cat. No. 1997). Centrifuge Samples (10000 x g, 4ºC, 10 min). Collect filtrate and discard retentate. **Cell lysate:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Nitrite Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. and transfer the supernatant to a fresh tube. Add 10-100 µl Sample per well & adjust the volume to 100 µl with Nitrite Assay Buffer.

   Notes:

   a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For Samples exhibiting significant background, prepare parallel Sample well(s) as Background Controls.

   c. To remove proteins, we suggest collecting ultrafiltrate from Samples on 10 kDa ultrafilters (Cat No.1997).

   d. Normal concentrations of Nitrite may vary, Urine: 1-20 µM; Serum: ~ 2 µM.

2. **Nitrite Standard Curve:** Dilute Nitrite Standard 100-fold by adding 5 µl of 100 mM Nitrite Standard to 495 µl Nitrite Assay Buffer to obtain 1 mM Nitrite Standard Solution. Add 0, 2, 4, 6, 8 and 10 µl of 1 mM Nitrite Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8, 10 nmol/well of Nitrite Standard. Adjust the volume to 100 µl/well with Nitrite Assay Buffer.

3. **Reaction Mix:** For each well, add the following reagents in the order as indicated in the table below. ***Do not premix Griess Reagents prior the experiment:***

	Reaction Mix	*Background Control Mix
Griess Reagent I	10 µl	10 µl
Griess Reagent II	10 µl	----
Nitrite Assay Buffer	80 µl	90 µl

   Add both Griess Reagents and Nitrite Assay Buffer separately to each well containing the Standard, Test Samples. Mix well. Incubate at RT for 10 min.

* For Background correction, add 100 µl of Background Control Mix to Sample Background Control well(s) and mix well.

4. **Measurement:** Measure absorbance at 540 nm in end-point mode at RT.

   Note: Signal is stable for one hour after Reaction Mix was added.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Nitrite Standard Curve. If Sample Background Control reading is significant, subtract the Background Control reading from its paired Sample reading. Apply the corrected ΔOD to the Nitrite Standard Curve to get B nmol of nitrite in the Sample well.

Sample Nitrite Concentration (C) = B/V x D = nmol/µl = mM

Where: **B** = Nitrite amount in the Sample from Standard Curve (nmol).
V = Sample volume added into the reaction well (ml).
D = Dilution Factor

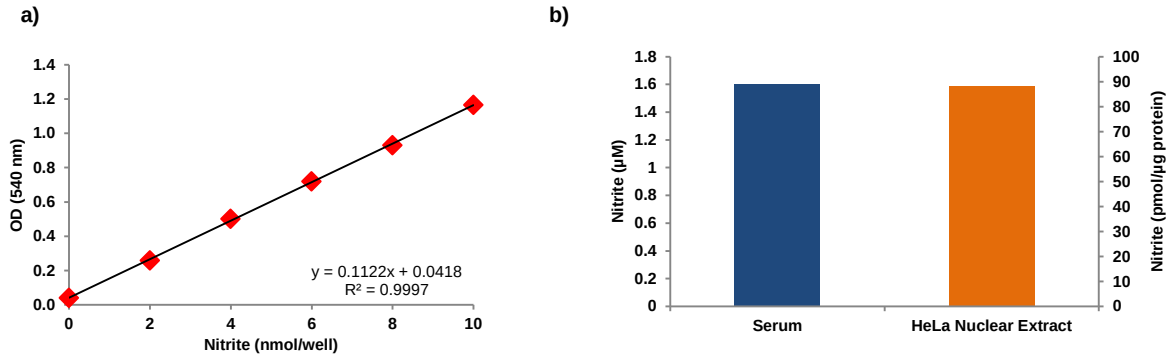

Figure: (a) Nitrite Standard Curve; **(b)** Nitrite concentration in Pooled human serum and HeLa nuclear extract; Human serum was deproteinized using 10 kDa columns. Filtrate (20 µl; undiluted) was collected and assayed according to kit protocols. HeLa nuclear cells were homogenized using Nitrite Assay Buffer (100 µg protein) were assayed as indicated.

VIII. RELATED PRODUCTS:

Nitric Oxide Colorimetric Assay Kit (K262)
Nitric Oxide Synthase (NOS) Activity Assay Kit (Colorimetric) (K205)
Nitric Oxide Synthase (NOS) Activity Assay Kit (Fluorometric) (K206)
Nitric Oxide Cell-Based HTS Assay Kit (K979)
Nitric Oxide Fluorometric Assay Kit (K252)
EZCell™ Intracellular Nitric Oxide Synthase (NOS) Detection Kit (K207)

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