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ab234044
Griess Assay Kit / Griess Reagent Kit

For the measurement of nitrite in biological samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Griess Assay Kit / Griess Reagent Kit (ab234044) utilizes a classic protocol for the estimation of nitrite in biological samples.

Nitrite is reduced to nitrogen oxide using Griess Reagent I. Nitrogen oxide then reacts with Griess Reagent II forming a stable product that can be detected by its absorbance at 540 nm. The two-step assay is simple, fast and can detect nitrite levels as low as 1 nmol/well.

Prepare standards, samples and controls as directed and add to plate.

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Add Reaction Mix to standards and samples in the specified order Griess Reagent I, Griess Reagent II, Buffer III

Do NOT premix

⇩

Add Background Control mix to control samples.

⇩

Incubate plate for 10 minutes at room temperature.

⇩

Read absorbance at 540 nm within 60 minutes.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temp (before prep)	Storage temp (after prep)
Buffer III	30 ml	-20°C	-20°C
Griess Reagent I	10 ml	-20°C	-20°C
Griess Reagent II	10 ml	-20°C	-20°C
Nitrite Standard	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 540 nm.
- 96 well plate with clear flat bottom

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Buffer III

Ready to use as supplied.

5.2 Griess Reagent I

Ready to use as supplied.

5.3 Griess Reagent II

Ready to use as supplied.

5.4 Nitrite Standard

1. Reconstitute with 100 µl Buffer III to generate 100 mM Nitrite Standard solution.
2. Keep on ice during use.

Δ Note: *Reconstituted standard is stable for 4 months when stored at 4°C.*

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute Nitrite Standard from Step 5.4 100-fold by adding 5 µl of 100 mM Nitrite Standard to 495 µl Buffer III to obtain 1 mM Nitrite Standard Solution.
2. Using the 1 mM Nitrite Standard Solution, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	1mM Nitrite Standard (µL)	Buffer III (µL)	Final volume standard in well (µL)	End amount Nitrite Standard in well (nmol/well)
1	0	200	100	0
2	4	196	100	2
3	8	192	100	4
4	12	188	100	6
5	16	184	100	8
6	20	180	100	10

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

7. Sample Preparation

7.1 Urine:

Dilute samples 10-fold using Buffer III.

7.2 Serum/Plasma:

1. De-proteinize samples using 10 kDa columns (ab93349).
2. Centrifuge samples (10,000 x g, 4 °C for 10 minutes).
3. Collect ultra-filtrate and discard retentate.

7.3 Cell Lysate:

1. Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Buffer III.
2. Keep on ice for 10 minutes.
3. Centrifuge at 10,000 x g for 5 minutes and transfer the supernatant to a fresh tube.

Δ Note: *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*

Δ Note: *Normal concentrations of nitrite may vary. Urine: 1-20 µM; Serum: ~ 2 µM.*

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction wells set up:

- Standard wells = 100 µl standard dilutions.
- Sample wells = 10-100 µl samples (adjust volume to 100 µl/well with Buffer III).
- Sample Background Control wells = 10-100 µl samples (adjust volume to 100 µl/well with Buffer III).

8.2 Reaction mix:

1. For each well, add the following reagents in the order indicated in the table below.

Δ Note: *Do NOT premix Griess Reagents prior the experiment.*

Component	Reaction Mix (µl)	Background Reaction Mix (µl)
Griess Reagent I	10	10
Griess Reagent II	10	---
Buffer III	80	90

2. Add both Griess Reagents and the Buffer III separately to each well containing the Standard, Test samples. Mix well.
3. Add 100 µl of Background Reaction Mix into the background control sample wells. Mix well.

Δ Note: *For samples exhibiting significant background, it is important prepare parallel sample well(s) as background controls.*

8.3 Measurement:

1. Incubate the plate for 10 minutes at room temperature.
2. Measure absorbance at 540 nm in end-point mode at room temperature.

Δ Note: *Signal is stable for one hour after addition of Reaction Mix.*

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (zero standard) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the Sample Background Control from sample readings.
4. Plot the corrected values for each standard as a function of the amount of nitrite to generate a standard curve.
5. Apply the corrected sample OD reading to the standard curve to get the Nitrite (B) amount in the sample wells (nmol/well).
6. Concentration of Nitrite in mM in the test samples is calculated as:

$$\text{Nitrite concentration} = \frac{B}{V} * D$$

Where:

B = amount of Nitrite in the sample well calculated from standard curve in [nmol].

V = sample volume added in the sample wells [ml].

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

10. Typical Data

Data provided **for demonstration purposes only.**

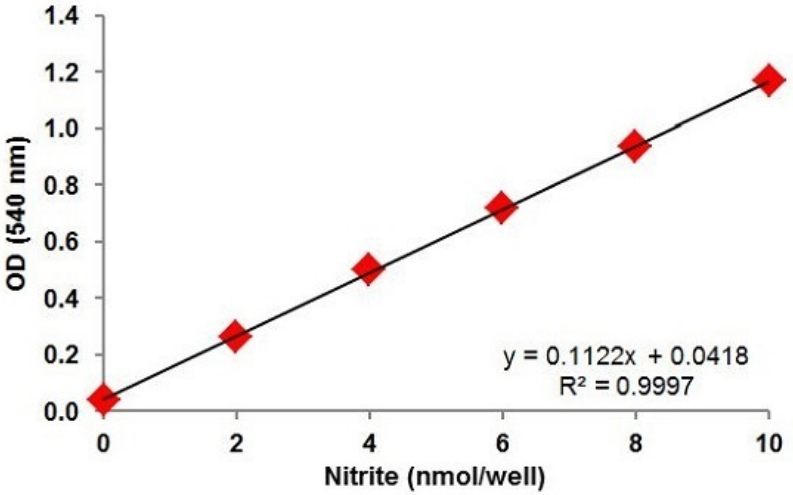

Figure 1. Nitrite standard curve.

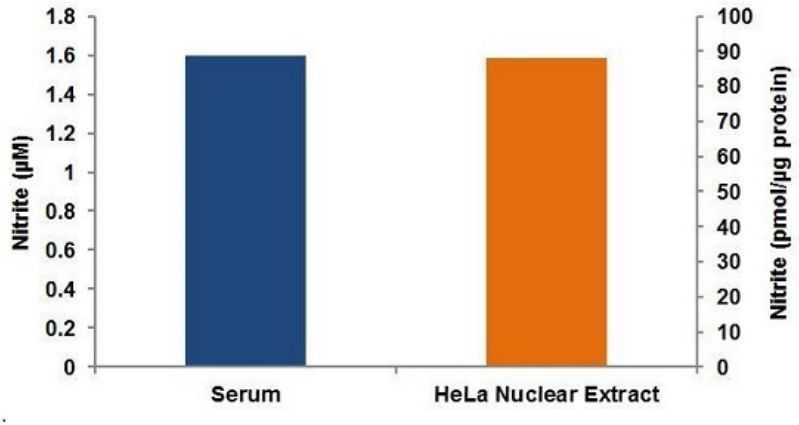

Figure 2: Nitrite concentration in pooled human serum and HeLa nuclear extracts. Human serum was deproteinized using 10 kDa columns. Filtrate (50 µl, undiluted) was collected and assayed according to kit protocols. HeLa nuclear cells were homogenized using Buffer III (100 µg protein).

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)