Product Manual

Nitric Oxide (NO_2/NO_3) detection kit

Catalog # ADI-917-010

192 Well (2x96) Kit

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Product Manual

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

Please contact Enzo Life Sciences Technical Support if necessary.

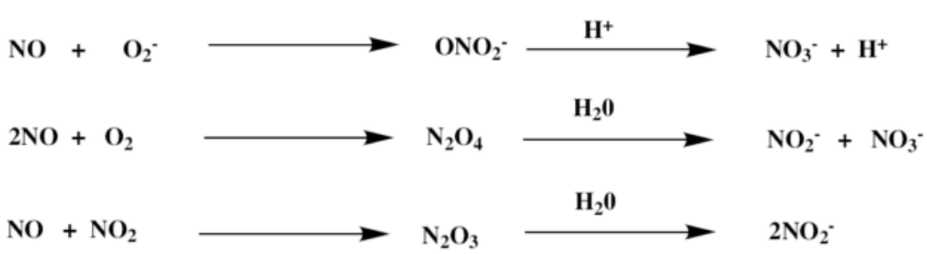
TABLE OF CONTENTS

DESCRIPTION

The Nitric Oxide (NO_2/NO_3) detection kit is a complete kit for the quantitative determination of nitrite and nitrate in biological fluids. Please read the complete kit insert before performing this assay. The kit involves the enzymatic conversion of nitrate to nitrite, by the enzyme Nitrate Reductase, followed by the colorimetric detection of nitrite as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm. The conversion of NO into nitrate and nitrite by the reactions outlined above, varies in each system and so both nitrite and nitrate concentrations should be measured. The interaction of NO in a system is measured by the determination of both nitrate and nitrite concentrations in the sample. The relative levels of nitrite and nitrate can vary substantially, therefore the most accurate determination of total nitric oxide production requires quantization of both nitrate and nitrite.

INTRODUCTION

Nitric oxide (NO) is a major mammalian secretory product that initiates host defense, homeostatic and developmental functions by either direct effect or intercellular signaling[1]. NO is the product of a five-electron oxidation of the amino acid L-arginine mediated by nitric oxide synthase[2]. As a direct effector, NO is thought to activate regulatory proteins, kinases and proteases that are directed by reactive oxygen intermediates[3]. As a messenger molecule, NO covalently interacts with target molecules based on redox potential rather than noncovalent complementarity[1]. Activation of the immune system is associated with an increase in macrophage NO production[4]. NO exerts a variety of homeostatic influences as an activator of soluble guanylyl cyclase[5], as a neuronal potentiator[6], a peripheral nervous system neurotransmitter[7], and a contraction regulator of both smooth muscle and vascular tissue[8]. In addition, NO has been linked to the formation of olfactory[9] and synaptic memories and remodeling[10]. The transient and volatile nature of NO makes it unsuitable for most convenient detection methods; however, two stable breakdown products, nitrate (NO_3) and nitrite (NO_2) can be easily detected by photometric means.



SAFETY WARNINGS & PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Handle with care

- The Griess Reagents I and II are dissolved in 2 M hydrochloric acid. CAUTION: Caustic; care should be taken in use. The Griess Reagents I and II contain organic chemicals. Care should be taken in handling these materials.
- The water used for any dilutions of samples should be free of endogenous nitrite and nitrate. We suggest the use of deionized or distilled water. Take care to avoid nitrate/nitrite contamination of samples or buffers. Care should be used in the selection of disposable gloves and transfer pipettes as sources of contamination. Please refer to reference 11 concerning nitrate and nitrite contamination in these products.
- Dispose of the contents of the plate with care. Attention should be taken in handling because of unknown effects of the contents.

MATERIALS PROVIDED

1. **Microtiter Plate, 2 Each, Catalog No. 80-0144**

   Break apart microtiter plates. The plates are ready to use.

2. **Nitrate Reductase, 1 Vial, Catalog No. 80-1347**

   A vial of lyophilized Nitrate Reductase, with desiccant. Store at -20°C.

3. **Nitrate Reductase Storage Buffer, 4 mL, Catalog No. 80-0255**

   A phosphate based buffer containing preservatives.

4. **Desiccated NADH , 2 Bottles, Catalog No. 80-0258**

   Vials of lyophilized reduced ß-Nicotinamide adenine dinucleotide, Catalog No. 80-0256. Store in the Dark.

5. **Nitrite Standard, 0.5 mL, Catalog No. 80-0224**

   A solution of sodium nitrite at 2,000 µmol/L in water with preservatives.

6. **Nitrate Standard, 0.5 mL, Catalog No. 80-0223**

   A solution of sodium nitrate at 1,000 µmol/L in water with preservatives.

7. **Reaction Buffer Concentrate, 30 mL, Catalog No. 80-0257**

   A HEPES-based buffer containing detergent and preservatives.

8. **Griess Reagent I, 12 mL, Catalog No. 80-0227**

   A solution of sulfanilamide in 2M hydrochloric acid.

9. **Griess Reagent II, 12 mL, Catalog No. 80-0228**

   A solution of N-(1-Naphthyl)ethylenediamine in 2M hydrochloric acid.

10. **Plate Sealer, Two, Catalog No. 30-0012**

11. **Nitric Oxide Assay Layout Sheet, One, Catalog No. 30-0218**

Reagents require separate storage conditions.

STORAGE

All components of this kit are stable at 4°C until the kit's expiration date, except the Nitrate Reductase enzyme which must be stored at -20°C prior to and after reconstitution.

OTHER MATERIALS NEEDED

1. Deionized or distilled water. No difference in assay results is seen with distilled water.
2. Precision pipets for volumes between 25 µL and 1,000 µL.
3. Repeater pipets for dispensing 25 µL and 50 µL.
4. Ice bath or refrigerated container capable of maintaining 0°C.
5. A 37°C Incubator.
6. Microplate reader capable of reading between 540-570 nm.
7. Linear graph paper for plotting the standard curve.
8. 10,000 MWCO polysulfone filters should be used. The end user should chose the specific 10,000 MWCO filter that best suits their sample volume needs.

INTERFERENCES

The Nitric Oxide assay is compatible with nitrite and nitrate in samples in a wide range of matrices. Samples diluted into Reaction Buffer or water can be read directly from the standard curve. The Griess reaction involves the interaction of nitrite ions with two organic molecules and involves an oxidation and nucleophilic reaction. Buffer or sample components that may interfere with this oxidation and nucleophilic reaction may interfere with color formation. The conversion of nitrate to nitrite involves the enzyme Nitrate Reductase. Any sample or buffer component that may interfere with this enzyme will lower the conversion of sample nitrate to nitrite and therefore give rise to lower estimates of NO.

Examples of nucleophiles and antioxidants that may interfere with the assays are azide, ascorbic acid, sulfhydryl containing compounds such as cysteine, glutathione, DTT and ß-mercaptoethanol. If concentrations of these materials are to be in excess of 10 µM in the sample, a test of nitrate recovery should be made using the nitrate provided with the kit. Nitrate at concentrations similar to those used for the standard curve should be added to the buffer containing the suspected interfering compound, and to a similar buffer without the suspected interfering compound. If there is a significant change in the nitrate concentrations found in the buffer containing the interfering compound the effect should be determined and suitable corrections made.

Some tissue culture media, such as RPMI, contain high nitrate concentrations and should not be used as this will interfere with sensitive detections. Media that contain phenol red as a pH indicator do not interfere with the Griess reaction as the indicator is typically yellow colored under the conditions of the Griess reaction. Certain systems involving NO synthetase enzymes utilize high concentrations (0.5-1 mM) of NADPH which may inhibit the Griess color reaction slightly. Care should be taken to ensure that these are diluted sufficiently (≥ 1:10) in Reaction Buffer to minimize any effect of NADPH. If samples may contain excessive amounts of NADPH this can be oxidized using Lactate Dehydrogenase (LDH) and pyruvic acid prior to color formation[12]. 10 µL of 1,500 U/mL LDH (Sigma, L-1378) in 30 mM sodium pyruvate (P-2256) is added to all wells after incubation with Nitrate Reductase and incubated at 37°C for 10 minutes prior to addition of the Griess reagents.

SAMPLE HANDLING

The dilutions recommended are to remove matrix interference in the assay. The optimal dilution for each experiment should be determined by the investigator.

Whole Organism Samples

In studies on isolated organisms the environmental nitrite and nitrate must be taken into account. Any media or fluid that the organism is stored in should be analyzed separately. Adjustments to the nitrite and nitrate in the organism must take into account the turnover of environmental nitrite and nitrate by the organism.

Urine

Fresh urine samples should be diluted at least 1:20 into Reaction Buffer, filtered through a 10,000 Molecular Weight Cut Off (MWCO) filter and used directly in the assay. If the samples need to be stored, either suitable antibiotics, such as penicillin or streptomycin at 100 U/mL, or 2-propanol at 6.5% (v/v) can be added prior to storing at -80°C.

Saliva

Saliva samples should be diluted 1:2 - 1:100 into Reaction Buffer, filtered through a 10,000 MWCO filter and used directly in the assay. Typical saliva samples may contain a relatively high concentration of nitrate which is thought to be produced by oral bacteria.

Plasma

Citrate plasma is recommended. Plasma should be used directly in the assay after a 1:2 - 1:20 dilution into Reaction Buffer and filtration through a 10,000 MWCO filter. EDTA or heparinized plasmas may be used in the assay after dilution into Reaction Buffer and filtration through a 10,000 MWCO filter, however they may not give reproducible results as the protein may precipitate during the Griess reaction.

Serum

Serum should be diluted 1:2 - 1:20 into Reaction Buffer filtered through a 10,000 MWCO filter and used directly in the assay.

Culture supernates

Avoid media containing nitrate salts. Samples should be diluted at least 1:2 in Reaction Buffer and filtered through a 10,000 MWCO filter and used directly in the assay.

PROCEDURAL NOTES

- Do not mix components from different lot numbers or use reagents beyond the expiration date.
- Allow all reagents, except the Nitrate Reductase enzyme, to warm to room temperature for at least 30 minutes before opening.
- Standards can be made up in either glass or plastic tubes; no difference in assay result is seen with either type of tube.
- Pre-rinse the pipet tip with the reagent and use fresh pipet tips for each sample, standard and reagent.
- Add the reagents to the side of the well to avoid contamination.
- The Nitrate Reductase enzyme must be kept at 0°C during use. The enzyme solution must be stored at -20°C both prior to use and after reconstitution.

REAGENT PREPARATION

1. **Reaction Buffer**

   Prepare the Reaction Buffer by diluting 10 mL of the supplied concentrate with 90 mL of deionized water. This can be stored at room temperature for 3 months.

2. **NADH Reagent**

   Reconstitute a NADH vial by adding 1 mL of water. Wait for 3 minutes and vortex prior to use. Use on ice. Store unused NADH solution tightly capped at -20°C. Stable at -20°C for 45 days. Final NADH Dilution. Prior to use take 0.9 mL of the NADH solution and add 1.8 mL of water. Vortex. Use on ice, and store tightly capped at -20°C. Stable at -20°C for 45 days. Avoid repeated freeze/thaw cycles.

3. **Nitrate Reductase Enzyme Reconstitution**

   Enzyme Dilution I: Reconstitute the Nitrate Reductase vial with 1 mL of Nitrate Reductase Storage Buffer. Vortex vigorously. Let sit at room temperature for 15 minutes. Vortex again. Let sit at room temperature for an additional 15 minutes. Vortex. Use on ice, and store tightly capped at -20°C. Stable at -20°C for 3 months. Avoid repeated freeze/thaw cycles. Final Enzyme Dilution: Count the total number of wells needed for the samples and add 14 (for the complete standard curve in duplicate). Use the

following formula to calculate the volume of Enzyme Dilution I and Reaction Buffer required for the Final Enzyme Dilution.

A. Volume of Enzyme Dilution I (µL) =

[Number of wells, incl. Standard Curve Wells + 2] x 10 µL

B. Volume of Reaction Buffer (µL) =

[Volume of Enzyme Dilution I in µL (from A. above)] x 1.5 µL

Immediately before use, pipet the volume of Enzyme Dilution I from A. above and add to it the volume of Reaction Buffer from B. above. Vortex and use on ice within 15 minutes.

4. Nitrite Standard

Allow the 2,000 µmol/L Nitrite standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 0.9 mL of Reaction Buffer into tube #1. Pipet 0.5 mL of Reaction Buffer into tubes #2 - #7. Add 100 µL of the 2,000 µmol/L standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7. The concentration of Nitrite in tubes #1 through #7 will be 200, 100, 50, 25, 12.5, 6.25, and 3.125 µmol/L respectively. See Nitric Oxide Assay Layout Sheet for dilution details.

5. Nitrate Standard

Allow the 1,000 µmol/L Nitrate Standard Solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 0.9 mL of Reaction Buffer into tube #1. Pipet 0.5 mL of Reaction Buffer into tubes #2 - #6. Add 100 µL of the 1,000 µmol/L standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #6. The concentration of Nitrate in tubes #1 through #6 will be 100, 50, 25, 12.5, 6.25, and 3.125 µmol/L respectively. See Nitric Oxide Assay Layout Sheet for dilution details.

Nitrite Assay Procedure

1. Determine the number of wells to be used.
2. All standards and samples should be run in duplicate.
3. Pipet 200 µL of Reaction Buffer into duplicate Blank wells.
4. Pipet 50 µL of Nitrite Standards #1 through #7 into duplicate wells.

5. Pipet 50 µL of Reaction Buffer into duplicate wells to act as a Zero Standard.
6. Pipet 50 µL of Samples into duplicate wells.
7. Pipet 50 µL of Reaction Buffer into the Zero Standard, Standards #1-#7 and Sample wells.
8. Pipet 50 µL of the Griess Reagent I into each well, except the Blank Wells.
9. Pipet 50 µL of the Griess Reagent II into each well, except the Blank Wells.
10. Mix well by shaking or tapping the side of the plate.
11. Incubate the plate at room temperature for 10 minutes.
12. Read the optical density of each well at 540 ± 20 nm after blanking against the Blank Wells.

Nitrate Assay Procedure

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2. All standards and samples should be run in duplicate.
3. Pipet 200 µL of Reaction Buffer into duplicate Blank wells.
4. Pipet 50 µL of Nitrate Standards #1 through #6 into duplicate wells.
5. Pipet 50 µL of Reaction Buffer into duplicate wells to act as a Zero Standard.
6. Pipet 50 µL of Samples into duplicate wells.
7. Pipet 25 µL of NADH into all Standard and Sample wells.
8. Pipet 25 µL of Nitrate Reductase Final Enzyme Dilution into all Standard and Sample wells.
9. Mix well and apply plate sealer to wells. Incubate for 30 minutes at 37°C.
10. Pipet 50 µL of the Griess Reagent I into each well, except the Blank Wells.
11. Pipet 50 µL of the Griess Reagent II into each well, except the Blank Wells.
12. Mix well by shaking or tapping the side of the plate.
13. Incubate the plate at room temperature for 10 minutes.
14. Read the optical density of each well at 540 ± 20 nm after blanking against the Blank wells.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of Nitrite or Total Nitrite and Nitrate in the samples.

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Zero Standard OD from the average OD for each standard and sample.

Average Net OD = Average OD - Average Zero Standard OD

2. Plot the Average Net OD for each Standard versus Nitrite or Nitrate Concentration.
3. Plot the Average OD for each Sample and extrapolate Nitrite or Total Nitrite and Nitrate concentration from the graph.
4. Subtract the Nitrite concentration from the Total Nitrite and Nitrate concentration to obtain the Nitrate concentration in the sample.

Notes on Calculation of Nitrite and Nitrate Concentration

This kit allows the user to determine the concentrations of Nitrite (NO2) and Nitrate (NO3) in the sample. In this first assay, the user is measuring just the Nitrite concentration. In the second assay, Nitrate is reduced to Nitrite, and the concentration of Nitrite is measured. By subtracting the result of the first assay (Nitrite) from the second assay (Total Nitric Oxide), the user can determine both the concentration of Nitrite and Nitrate in a given sample.

1. Use the standard curve from the Nitrite Assay to interpolate the concentration of Nitrite (NO_2). Call this data point (i), which will have units of µmol/L. (i) will represent the amount of nitrite in the sample.
2. Use the standard curve from the Nitrate Assay to interpolate the Total Nitric Oxide concentration (NO_2 + NO_3) in the sample (Total Nitrite in sample before and after Nitrate reduction). Call this data point (a), which will also have units of µmol/L.
3. To calculate the concentration of Nitrate (NO_3), subtract nitrite concentration in sample (i) determined in step 1 from total nitric oxide concentration in sample (a) determined in step 2.

Nitrate (NO_3) concentration in sample = (a)-(i)

To calculate the final concentration of Nitrite or Nitrate in your sample just multiply by the dilution factor used in preparing the samples.

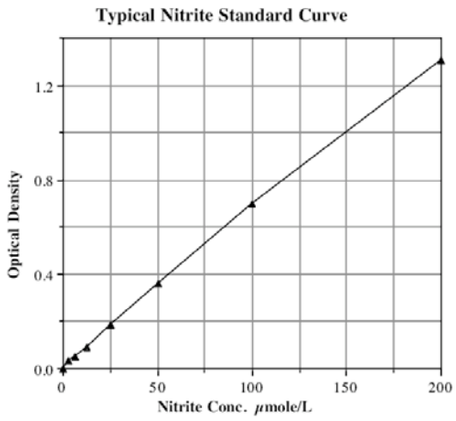
TYPICAL RESULTS

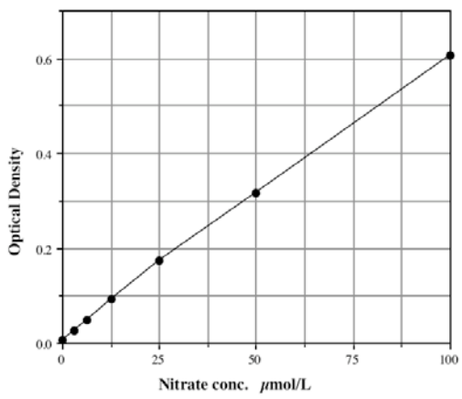
The results shown below are for illustration only and should not be used to calculate results from another assay.

NITRITE ASSAY				NITRATE ASSAY			
Sample	**Average OD**	**Net OD**	**Nitrite µmol/L**	**Sample**	**Average OD**	**Net OD**	**Nitrate µmol/L**
Blank	0.000			Blank	0.038	0.000	
S1	1.310	1.303	200	S1	0.644	0.606	100
S2	0.705	0.698	100	S2	0.355	0.317	50
S3	0.367	0.360	50	S3	0.212	0.174	25
S4	0.190	0.183	25	S4	0.132	0.094	12.5
S5	0.099	0.092	12.5	S5	0.086	0.048	6.25
S6	0.059	0.052	6.25	S6	0.066	0.028	3.125
S7	0.040	0.033	3.125	__	___	___	___
Zero	0.007	0.000	0	Zero	0.046	0.008	0

TYPICAL STANDARD CURVES

Typical standard curves are shown below. The curves must not be used to calculate Nitrite or Nitrate concentrations; each user must run a standard curve for each assay.





PERFORMANCE CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[12].

Nitrate and Nitrite Sensitivity

Nitrite sensitivity was calculated by determining the average optical density for sixteen (16) wells run as the zero standard, and comparing to the average optical density for sixteen (16) wells run with Standard #7. Nitrate sensitivity was calculated by determining the average optical density for twenty-four (24) wells run as the zero standard, and comparing to the average optical density for twenty-four (24) wells run with standard #6. The detection limit was determined as the concentration of Nitrite or Nitrate measured at two (2) standard deviations from the zero along the standard curve.

Nitrite Sensitivity

OD for Zero Standard =
0.0123 ± 0.0011 OD for Standard #7 = 0.0433 ± 0.0018

Delta Optical Density (3.125 - 0 µmol/L) =
0.0433 - 0.0123= 0.0310 2 SD's of S0 = 0.0022

$$\text{Sensitivity} = \frac{0.0022}{0.0310} \times 3.125\ \mu\text{mol/L} = 0.222\ \mu\text{mol/L}$$

Nitrate Sensitivity

OD for Zero Standard =
0.008 ± 0.004 OD for Standard #6 = 0.028 ± 0.002.

Delta Optical Density (3.125 - 0 µmol/L) =
0.028 - 0.008= 0.020 2 SD's of S0 = 0.0040.

$$\text{Sensitivity} = \frac{0.0040}{0.020} \times 3.125\ \mu\text{mol/L} = 0.625\ \mu\text{mol/L}$$

Linearity

Nitrite Assay

A sample containing 125 µmol/L Nitrite was serially diluted 6 times 1:2 in the buffer supplied in the kit and measured in the Nitrite assay. The data was plotted graphically as actual Nitrite concentration versus measured Nitrite concentration. The line obtained had a slope of 1.0230 with a correlation coefficient of 0.9995.

Nitrate Assay

A sample containing 75 µmol/L Nitrate was serially diluted 6 times 1:2 in the buffer supplied in the kit and measured in the Nitrate assay. The data was plotted graphically as actual Nitrate concentration versus measured Nitrate concentration. The line obtained had a slope of 1.0595 with a correlation coefficient of 0.9995.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of Nitrite and Nitrate and running these samples multiple times (n=8) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of Nitrite and Nitrate in multiple assays (n=8). The precision numbers listed below represent the percent coefficient of variation for the concentrations of Nitrite and Nitrate determined in these assays as calculated by a linear curve fitting program.

	Nitrite (µM/L)	**Inter Assay (%CV)**	**Intra Assay (%CV)**	**Nitrate (µM/L)**	**Inter Assay (%CV)**	**Intra Assay (%CV)**
Low	7.67	7.7		10.49	3.4	
Medium	25.75	2.9		16.93	2.8	
High	64.61	1.3		21.93	5.5	
Low	8.03		2.4	8.71		1.5
Medium	25.11		1.7	19.20		1.2
High	64.14		0.8	25.45		0.8

NITRITE AND NITRATE SAMPLE RECOVERIES

Nitrite and Nitrate concentrations were measured in a variety of different samples. Nitrite and Nitrate were spiked into samples which had been diluted with the kit Reaction Buffer. The diluted samples were filtered through a 10,000 MWCO filter and assayed in the kit. The following results were obtained:

	Nitrite Assay		Nitrate Assay*	
Sample	**% Recovery**	**Rec. Dilution****	**% Recovery**	**Rec. Dilution****
Tissue Culture Media	100	1:2	88	1:2
Human Saliva	96	1:2	100	1:2-1:100
Human Urine	110	1:2	104	1:20
Human Serum	112	1:2	93	1:2-1:20
Citrate Plasma	118	1:2	98	1:2-1:20
Human EDTA Plasma	89	1:2	91	1:2-1:20
Heparin Plasma	117	1:2	96	1:2-1:20
Reaction Buffer	117	1:2	88	1:2

*Please note that the recommended dilutions for the Nitrate Assay take into account the normal levels of Nitrate in some samples.

**See Sample Handling instructions on Page 7 for full details.

REFERENCES

1. C. Nathan, FASEB J., (1992) 6: 3051-3064.
2. S. Moncada, et al., Biochem. Pharm., (1989) 38(11): 1709-1715.
3. R. Schreck and P.A. Baeuerle, Trends in Cell Biology., (1991) 1: 39-42.
4. D.J.Stuehr, and M.A. Marletta, PNAS., (1985) 82: 7738-7742.
5. L.J. Ignarro, Pharmac. & Toxicol., (1990) 67: 1-7.
6. D.S. Brendy, et al., Nature, (1990) 347: 768-770.
7. J. Garthwaite, Trends in Neuroscience, (1991) 14: 60-67.
8. E.M. Conner and M.B. Grisham, Methods, (1995) 7: 3-13.
9. K.M. Kendrich, et al., Nature, (1997) 388: 670-674.
10. R. Nowad, J. NIH Res., (1992) 4: 49-55.
11. S. Makela, et al., Clin. Chem., (1997) 43: 2418-2420.
12. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

NOTES

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