DeQuanto® Nitric Oxide ELISA KIT

QT4098

USER MANUAL

Immunoassay for quantitative determination of Nitric Oxide

50- Test

Research Use Only (RUO)

Please read this user's manual carefully before using the kit

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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INTENDED USE

The kit is a nitrate reductase method for the in vitro quantitative measurement of NO in blood serum, gastric juice, urine, cell culture supernates and other biological fluids.

NO has active chemical property, it is converted to nitrate (NO3-) & nitrite (NO2-). As result, it needs amount of blood serum NO3- content plus blood serum NO2- content to express NO level in vivo accurately. Some domestic units use metal cadmium (Cd) reduction method, but this method has tedious operations (protein in blood serum must be removed), low-controllable reactions (cadmium may reduce NO2-further), low determinacy (cadmium cannot reduce NO3- to NO2-completely), low accuracy.

Compared with metal cadmium method, nitrate reductase method is a sensitive, convenient, fast, stable, easy-generalized method.

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity(50/25T)	Reagents	Quantity(50/25T)
Reagent 1	2×6ml/1×6ml	Reagent 6	1/1
Reagent 2	2×6ml/1×6ml	Reagent 7	1×8mL//1×4ml
Reagent 3	1×12ml//1×6ml	Standard(10mmol/L	1×0.5ml/1×0.5ml
Reagent 4	1×6ml//1×3ml	Attachment	2×40ml/1×40ml
Reagent 5	1/1	Instruction manual	1/1

Note ：Attachment is double distilled water.

MATERIALS REQUIRED BUT NOT SUPPLIED

A spectrophotometer capable of measuring absorbance at 550nm, glass cuvettes of 0.5cm light path

2. Thermostatic water bath or air bath capable of controlling temperature at 37℃ and 90~100℃
3. Desk centrifuge,
4. Micropipets and tips
5. Vortex mixer, glass rod
6. A source of pure water (preferably double distilled water and double distilled water)

STORAGE OF THE KITS

1. Reagent 1: Can be stored at -20℃ or even colder for 3 months. Please place bottle in 37℃ or at room temperature to dissolve completely before use.
2. Reagent 2: Can be stored at -20℃ or even colder for 3 months. Please place bottle in 37℃ or at room temperature to dissolve completely before use.

3. Reagent 3: Can be stored at room temperature for 12 months.
4. Reagent 4: Can be stored at room temperature for 12 months.
5. Reagent 5: Can be stored away from light.
6. Reagent 6: Can be stored by cold preservation away from light. If its color becomes dark brown, then please discard it.
7. Reagent 7: Can be stored at room temperature for 12 months.
8. 10mmol/L standard: Can be stored at -20℃ or even colder for 12 months.

REAGENT PREPARATION

1. Mixed reagent preparation: Mix Reagent 1 and Reagent 2 at ratio of 1:1, how much you need, how much you prepare. After mixing completely, mixed reagent is available in 24 hours.
2. 2. Reagent 5: Powder×1 vial, when use, add 20ml*(50T) or 10ml*(25T) 90℃ ~100℃ hot distilled water to dissolve completely, can be stored away from light. Reagent 5 is supersaturated solution, it is better to add 23ml(50T)/11.5ml(25T) hot distilled water (consider thermal expansion), adding, heating by water bath & stirring by glass rod in same time to dissolve completely. There may be crystals in old Reagent 5 (from last experiment), please heat and stir it to dissolve completely.
3. 3. Reagent 6: Powder×1 vial, when use, add 8ml*(50T) or 4ml*(25T) double distilled water to dissolve it. Can be stored by cold preservation away from light. If its color becomes dark brown, then please discard it.
4. 4. Chromogenic agent: How much you need, how much you make (if you can exhaust chromogenic agent in 1 month, then you can prepare it in 1 time). Mix Reagent 5, Reagent 6 & Reagent 7 at ratio of 2.5:1:1. Can be stored in 40ml clean square bottle away from light. There are some crystals seed out in cold days, so when you use it again, please place it in 100℃ water bath, dissolve completely by shaking repeatedly.
5. 5. 100μmol/L standard working solution preparation: Take 0.1ml standard, add double distilled water until volume reaches to 10ml (100 times dilution), mix sufficiently, 100 μmol/L standard working solution is prepared. This working solution should be used soon after preparation.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.
4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for one pipetting.
5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be **used only once**.
6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

SAMPLE PREPARATION

1. Liquid sample (such as blood serum, gastric juice, urine, cell culture supernates and other biological fluids,etc.) NO assay: Pretreatment: According to Experimental Methodology.
2. Tissue sample NO assay : Pretreatment: According to Experimental Methodology.

ASSAY PROCEDURE

Liquid sample NO assay

Operation table:

	Blank tube	Standard tube	Sample tube
Double distilled (ml)	0.1		
100μmol/L standard working solution (ml)		0.1	
Liquid sample (ml)			0.1
Mixed reagent (ml)	0.4	0.4	0.4
Mix sufficiently, place in 37℃ water bath for 60 minutes accurately			
Reagent 3 (ml)	0.2	0.2	0.2
Reagent 4 (ml)	0.1	0.1	0.1
Mix sufficiently by vortex for 30 seconds, place quescently for 40 minutes, centrifugate at 3500~4000 rpm for 10 minutes, take supernatant for chromogenic reaction.			
Supernatant (ml)	0.5	0.5	0.5
Chromogenic agent(ml)	0.6	0.6	0.6
Mix sufficiently, place quescently for 10 minutes, transfer in cuvettes of 0.5cm light path, measure OD values of all tubes at 550nm (adjust zero by distilled water)			

Note: If you do this assay in winter, then please prewarm all reagents at 37℃ for 5 minutes.

b. If you have not enough supernatant, please extend centrifugation time length or take less supernatant (such as 0.4ml or 0.45ml), but please make sure that all tubes in one batch have sample sample volume. Never add sediment in tubes.

2. Tissue sample NO assay

Operation table:

	Blank tube	Standard tube	Sample tube
Double distilled (ml)	0.5	0.4	
100μmol/L standard working solution (ml)		0.1	
Homogenate (ml)			0.1

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Mixed reagent (ml)	0.4	0.4	0.4
Mix sufficiently, place in 37℃ water bath for 60 minutes accurately			
Reagent 3 (ml)	0.2	0.2	0.2
Reagent 4 (ml)	0.1	0.1	0.1
Mix sufficiently by vortex for 30 seconds, place quescently for 40 minutes, centrifugate at 3500~4000 rpm for 10 minutes, take supernatant for chromogenic reaction.			
Supernatant (ml)	0.8	0.8	0.8
Chromogenic agent(ml)	0.6	0.6	0.6
Mix sufficiently, place quescently for 10 minutes, transfer in cuvettes of 0.5cm light path, measure OD values of all tubes at 550nm (adjust zero by distilled water)			

Note:

a. If you do this assay in winter, then please prewarm all reagents at 37℃ for 5 minutes.

b. If you have not enough supernatant, please extend centrifugation time length or take less supernatant (such as 0.4ml or 0.45ml), but please make sure that all tubes in one batch have sample sample volume. Never add sediment in tubes.

TEST PRINCIPLE

NO has active chemical property, it is converted to nitrate (NO3 -) & nitrite (NO2-), NO2- can also convert to NO3-The method use nitrate reductase to reduce NO3- to NO2- selectively, calculate NO content by measuring OD values.

REFERENCED SAMPLE VALUE

1.Optimal sampling volumes:

Bovine blood serum: 300μl

Sheep blood serum: 300μl

Rat & mouse blood serum: 100μl

Rabbit blood serum: 100μl

10% tissue homogenate: 500μl

Cell suspension: 500μl

Cell culture supernates: 100μl

2.Normal NO contents:

Sample	Referenced sampling volume	Referenced NO content
Rat blood serum	100μl	38.0±23.4μmol/L
Mouse blood serum	100μl	49.96±23.76μmol/L
Rabbit blood serum	100μl	99.16±39.5μmol/L
Dog blood serum	100μl	89.51±32.13μmol/L

IMPORTANT NOTE

Please avoid free-thawing Reagent 1, Reagent 2 & standard repeatedly. If you want to do experiments in batches (>3), then you can subpack and store Reagent 1, Reagent 2 & standard when you do first experiment, when you do other experiment, take this reagent according to how much you use. Prepared chromogenic agent should be stored away from light.

2. If you don't use samples (such as blood serum, blood plasma, tissues, etc.) immediately, then you can store them at -70℃ or even lower temperature. Deep freezed samples are available in half year.

3. All reagent preparations and blank tubes need NO2--free double or triple distilled water (This kit includes double distilled water).

4. After complete reaction and centrifugation, please avoid suck sediment when you take supernatant, or OD values will increase largely, validity of results will be disturbed seriously

5. Test tube selection:

a. It is suggested to use disposable plastic test tubes (preferably disposable plastic test tubes from our institute).

b. If you want to use glass test tubes, then please soak them in detergent solution for more than half hour, boil for 0.5~1 hour, brush carefully, rinse by tap water for 15~20 times, discard water, rinse by distilled water for 1~2 times, oven dry them.

CUSTOMIZED SERVICES

PRECLINICAL/CLINICAL SAMPLE ANALYSIS
ELISA based validation and clinical or pre-clinical sample analysis.

POLYCLONAL ANTIBODY DEVELOPMENT
PAb development against peptide or protein, peptide designing, high titer antibody purification, characterization.

MONOCLONAL ANTIBODY DEVELOPMENT
MAb development against peptide or protein, peptide designing, best reacting/stable hybridoma development, sub cloning, small scale/large scale hybridoma culture and antibody purification, characterization.

CLONING, PROTEIN EXPRESSION PURIFICATION
Gene synthesis, site directed mutagenesis, cloning and expression in bacterial systems and other customized molecular biology services.

ANTIBODY CONJUGATION
With HRP, FITC, Biotin, ALP and many more molecules.

CELL BASED ASSAYS
Cell based assays, neutralization assays and potency ratio estimation of drugs with reference drugs, drug efficacy assessment and customized in-vitro, in-vivo assay development.

CUSTOMIZED ELISA BASED ASSAY/METHOD DEVELOPMENT
Different formats of ELISA based assay development.

For any queries/enquiries related to our products or services, please contact us by mailing us at info@denovobiolabs.com or call us at (+91) 80 29575711

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