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

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INTENDED USE

The kit is a ABTS method for the in vitro quantitative measurement of total antioxidant capacity within serum, plasma, tissue homogenate, cells (or cell culture supernates).

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity(96T)	Reagents	Quantity(96T)
Reagent 1	1×20ml	Reagent 4	1×0.2ml
Reagent 2	1×1ml	Reagent 5	1×0. 1ml
Reagent 3	1×0.5ml	96-well strip plate	1
Instruction manual	1/1		

MATERIALS REQUIRED BUT NOT SUPPLIED

Plate readers with light filters of desired wavelength (405-425nm).

STORAGE OF THE KITS

Reagent 1: Buffer Solution. Can be stored at -20C for 12 months.

2. Reagent 2: ABTS Solution. Can be stored at -20C for 12 months. Avoid Illumination.
3. Reagent 3: H2O2 Stock Solution. Can be stored at -20C for 12 months.
4. Reagent 4: Peroxidase Stock Solution. Can be stored at -20C for 12 months.
5. Reagent 5: 10mM Trolox Solution. Can be stored at -20C for 12 months. Avoid Illumination.

REAGENT PREPARATION

1. **Reagent 3 Solution Preparation:** Dilute the stock solution with double distilled water (DDW) to 40 times of its original volume.
2. **ABTS Solution Preparation:** Blend Reagent 1, Reagent 2 and Reagent 3 solution with the ratio of 76:5:4 in order to prepare the desired amount of ABTS solution. ABTS Solution should be preserved at RT without light and used within 30 min.
3. **Reagent 4 Solution Preparation:** Dilute the given reagent 4 with reagent 1 to the 10 times of its original volume. Reagent 4 solution should be prepared right before its usage with the amount needed.

SAMPLE PREPARATION

1. Aqueous Sample like Serum/Plasma

Pretreatment: Measure directly. Note, for plasma samples, EDTA is not a recommended coagulant.

2. Tissue Sample

Pretreatment: Weight the sample precisely and add the saline with ratio of 1g sample with 9ml saline. Homogenize the mixture in the ice water bath and the centrifuge the homogenate at 4C, 12000 rpm for 5min. Extract the supernatant for further measurement.

3. Cells

Pretreatment: Collect no less than 1 million cells and add 200 µl cold phosphate buffer solution. Disrupt the cells either by homogenization or sonication, and then centrifuge the homogenate at 4C, 12000 rpm for 5min. Extract the supernatant for further measurement.

Note: For tissue sample and cells, calculation requires the protein concentration of the homogenate. It is recommended to use our BCA Protein Quantification Kit to determine the protein concentration.

ASSAY PROCEDURE

Operation table:

	Blank	Standard	Sample
Distilled Water (ul)	10		
Trolox Solution (ul)		10	
Sample (ul)			10
Reagent 4 Solution (ul)	20	20	20
ABTS Solution (ul)	170	170	170
React at RT for 6 min. Read the optical density (OD) at 414nm with a plate reader.			

TEST PRINCIPLE

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) can be oxidized to greenish $ABTS^{+}$ in the presence of proper oxidants. The production of $ABTS^{+}$ can be inhibited with antioxidants and thus the total antioxidant capacity can be calculated based on the optical density of $ABTS^{+}$ at 414 or 734nm. Trolox is a water-soluble analog of vitamin E with the similar anti-oxidative capability and is applied as the antioxidant capacity equivalency.

$$ABTS^{\cdot+} \xrightarrow{\text{Anti oxidant}} ABTS$$

CALCULATION OF RESULTS

The total antioxidant capacity of the sample is calculated based on the standard curve made. From the OD values of the sample, the Trolox equivalent antioxidant capacity of the pretreated sample can be obtained. For tissue sample and cells

$$\frac{T-AOC}{mM/mgprot} = \frac{T-AOC}{mM/ml}(Pretreated) \div \frac{C_{pr}}{mgprot/ml}$$

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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