Datasheet for ABIN417447

NADPH Oxidase 4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	NADPH Oxidase 4 (NOX4)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.15 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.15 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of NOX4 in Tissue Homogenate,Cell Lysate,Biological Fluids
Sample Type:	Cell Lysate, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4). No significant cross-reactivity or interference between Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4) and analogues was observed.
Sensitivity:	0.057 ng/mL

Product Details

Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A • Detection Reagent B • Assay Diluent A • Assay Diluent B • Reagent Diluent (if Detection Reagent is lyophilized) • TMB Substrate • Stop Solution • Wash Buffer (30 x concentrate) • Instruction manual

Target Details

Target:	NADPH Oxidase 4 (NOX4)
Alternative Name:	NOX4 (NOX4 Products)
UniProt:	Q9NPH5
Pathways:	Carbohydrate Homeostasis, Smooth Muscle Cell Migration

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader

	with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the

	color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4) in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	1. Bring all kit components and samples to room temperature (18-25 °C) before use. 2. Standard - Reconstitute the Standard with 0.5 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 10 ng/mL. Prepare 7 tubes containing 0.25 mL Standard Diluent and produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, 0.156 ng/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0 ng/mL. 3. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and B, respectively (1:100). 4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x). 5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. 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. 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 once pipetting. 4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once. 5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved. 6. Contaminated water or container for reagent preparation will influence the detection result.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4 (NOX4) were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV<10%

Application Details

	Inter-Assay: CV<12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.
Expiry Date:	6 months

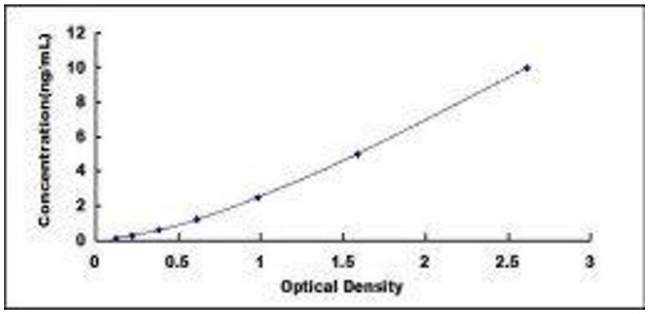
Publications

Product cited in:	Chakraborti, Pramanick, Saha, Sarkar, Singh, Stewart, Maity: "Biphasic changes in TGF-β1 signaling drive NSAID-induced multi-organ damage." in: **Free radical biology & medicine**, Vol. 160, pp. 125-140, (2020) (PubMed). He, Li, Luo, Li, Zhao, Qi, Liu, Yu: "Galectin-3 mediates the pulmonary arterial hypertension-induced right ventricular remodeling through interacting with NADPH oxidase 4." in: **Journal of the American Society of Hypertension : JASH**, Vol. 11, Issue 5, pp. 275-289.e2, (2017) (PubMed).

Publications

Vital, Castro, Ittmann: "Oxidative stress promotes benign prostatic hyperplasia." in: **The Prostate**, Vol. 76, Issue 1, pp. 58-67, (2016) (PubMed).

Images



ELISA

Image 1.