Datasheet for ABIN578904

# NID2 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | NID2 |
| Reactivity: | Rat |
| Method Type: | Sandwich ELISA |
| Application: | ELISA |

## Product Details

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| Purpose: | This immunoassay kit allows for the in vitro quantitative determination of rat PI3K concentrations in tissue homogenates and other biological fluids. |
| Sample Type: | Tissue Homogenate |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This assay recognizes recombinant and natural rat PI3K. |
| Cross-Reactivity (Details): | No significant cross-reactivity or interference was observed. |
| Sensitivity: | The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero. |
| Characteristics: | Rattus norvegicus,Rat,Nidogen-2,NID-2,Nid2 |
| Components: | Reagent (Quantity ): Assay plate (1), Standard (2), Sample Diluent (1 × 20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1 × 120µl), Detection Reagent B (1 × 120µl), Wash Buffer (25 x concentrate) (1 × 30ml), Substrate (1x10ml), Stop Solution (1x10ml), Plate sealer for 96 wells (5), Instruction (1) |

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## Product Details

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| Material not included: | Luminometer. Pipettes and pipette tips. EP tube Deionized or distilled water. |

## Target Details

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| Target: | NID2 |
| Alternative Name: | Nid2 (NID2 Products) |
| Background: | Phosphoinositide 3-kinases (PI 3-kinases or PI3Ks) are a family of related enzymes that are capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns). PI3Ks interact with the IRS (Insulin receptor substrate) in order to regulate glucose uptake through a series of phosphorylation events. The phosphoinositol-3-kinase family is composed of Class I, II and Class III, with Class I the only ones able to convert PI(4,5)P2 to PI(3,4,5)P3 on the inner leaflet of the plasma membrane. PI 3-kinases have been linked to an extraordinarily diverse group of cellular functions, including cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. Many of these functions relate to the ablilty of class I PI 3-kinases to activate protein kinase B (PKB, aka Akt). The class IA PI 3-kinase p110 alpha is mutated in many cancers. Many of these mutations cause the kinase to be more active. The PtdIns(3,4,5)P3 phosphatase PTEN which antagonises PI 3-kinase signalling is absent from many tumours. Hence, PI 3-kinase activity contributes significantly to cellular transformation and the development of cancer. The p110 δ and p110 gamma isoforms regulate different aspects of immune responses. PI 3-kinases are also a key component of the insulin signaling pathway. Hence there is great interest in the role of PI 3-kinase signaling in Diabetes mellitus. AKT is activated as a result of PI3-kinase activity, because AKT requires the formation of the PtdIns(3,4,5)P3 (or ",PIP3",) molecule in order to be translocated to the cell membrane. At PIP3, AKT is then phosphorylated by another kinase called PDK1, and is thereby activated. The ",PI3-k/AKT", signaling pathway has been shown to be required for an extremely diverse array of cellular activities - most notably cellular proliferation and survival. In addition to AKT and PDK1, one other related serine threonine kinase is bound at the PIP3 molecule created as a resulte of PI3-kinase activity, SGK. 2 |
| Gene ID: | 3179 |

## Application Details

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| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Protocol: | The microtiter plate provided in this kit has been pre-coated with an antibody specific to PI3K. |

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| | Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for PI3K. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain PI3K, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm 2 nm. The concentration of PI3K in the samples is then determined by comparing the O.D. of the samples to the standard curve. |
| Reagent Preparation: | Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 ml of Sample Diluent. This reconstitution produces a stock solution of 1,000 pg/mL. Allow the standard to sit for about 10 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the highest standard (1,000 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL). pg/mL 1,000 500 250 125 62.5 31.2 15.6 0 Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A or B (1:100), respectively. |
| Sample Collection: | Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 20 minutes at approximately 1000 g. Remove serum and assay immediately or aliquot and store samples at -20 or -80 . Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 g at 2 - 8 within 30 minutes of collection. Store samples at -20 or -80 . Avoid repeated freeze-thaw cycles. Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 or -80 . Avoid repeated freeze-thaw cycles. Note: Serum and plasma to be used within 7 days may be stored at 2-8, otherwise samples must stored at -20 ( 1 month) or -80 ( 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. |
| Assay Procedure: | Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of |

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the standard curve, users must determine the optimal sample dilutions for their particular experiments.

1. Add 100 of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for two hours at 37 .

2. Remove the liquid of each well, don ' t wash.

3. Add 100 µ l of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears 4 uniform.

4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µ l) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µ l of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for one hour at 37 .

6. Repeat the aspiration/wash process for five times as conducted in step

4. 7. Add 90 µ l of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 - 30 minutes at 37 . Protect from light.

8. Add 50 µ l of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Important Note:1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µ l for once pipetting.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

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## Application Details

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| | 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.<br>6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.<br>7. Duplication of all standards and specimens, although not required, is recommended.<br>8. Substrate Solution is easily contaminated. Please protect it from light. |
| Calculation of Results: | Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-23 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | 1. The kit should not be used beyond the expiration date on the kit label.<br>2. Do not mix or substitute reagents with those from other lots or sources.<br>3. If samples generate values higher than the highest standard, further dilute the samples and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding.<br>4. This assay is designed to eliminate interference by soluble receptors, ligands, binding 3 proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.<br>5. Limited by the current condition and scientific technology, we can't 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. |
| Storage: | 4 °C/-20 °C |
| Storage Comment: | The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be |

stored at -20 °C upon being received. The other reagents can be stored at 4 °C.

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