Datasheet for ABIN956014

# BNIP3 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | BNIP3 |
| Reactivity: | Rat |
| Method Type: | Sandwich ELISA |
| Detection Range: | 0.156-10 ng/mL |
| Minimum Detection Limit: | 0.156 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of rat BNIP3 in tissue homogenates and other biological fluids. |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This assay has high sensitivity and excellent specificity for detection of rat BNIP3. No significant cross- reactivity or interference between rat BNIP3 and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us - complete the cross-reactivity detection between rat BNIP3 and all the other analogues, therefore, cross reaction may still exist. |
| Sensitivity: | The minimum detectable dose of rat BNIP3 is typically less than 0.042 ng/mL. The Sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. |

## Product Details

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| | Value of 20 replicates of the zero calibrator plus three standard deviations. |
| Characteristics: | The microtiter plate provided in this kit has been pre-coated with an antibody specific to BNIP3. Calibrators or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for BNIP3. 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 BNIP3, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm +/- 10 nm. The concentration of BNIP3 in the samples is then determined by comparing the O.D. of the samples to the calibration curve. |
| Components: | Pre-coated, ready to use 96-well strip plate (1x)<br>Calibrator (lyophilized) (2x)<br>Calibrator Diluent (1 x 20 mL)<br>Detection Reagent A (1 x 120 µL)<br>Detection Reagent B (1 x 120 µL)<br>Assay Diluent A (2X concentrate) (1 x 6 mL)<br>Assay Diluent B (2X concentrate) (1 x 6 mL)<br>TMB Substrate (1 x 9 mL)<br>Stop Solution (1 x 6 mL)<br>Wash Buffer (30X concentrate) (1 x 20 mL)<br>Plate sealer for 96 wells (4x). |
| Material not included: | 1. Microplate reader with 450 +/- 10 nm filter.<br>2. Precision single and multi-channel pipettes and disposable tips.<br>3. Eppendorf Tubes for diluting samples.<br>4. De-ionized or distilled water.<br>5. Absorbent paper for blotting the microtiter plate.<br>6. Container for Wash Solution. |

## Target Details

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| Target: | BNIP3 |
| Alternative Name: | BCL/Adenovirus E1B 19kDa Interacting Protein 3 (BNIP3) (BNIP3 Products) |
| Pathways: | Autophagy, Brown Fat Cell Differentiation |

## Application Details

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| Comment: | The calibration curve concentrations used for the ELISA's were 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. |
| Plate: | Pre-coated |
| Assay Procedure: | 1. Determine wells for diluted calibrator, blank and sample. Prepare 7 wells for calibrator, 1 well for blank. Add 100 µL each of dilutions of calibrator (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37° C.<br>2. Remove the liquid of each well, don't wash.<br>3. Add 100 µL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37° C after covering it with the Plate sealer.<br>4. Aspirate the solution and wash with 350 µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.<br>5. Add 100 µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37° C after covering it with the Plate sealer.<br>6. Repeat the aspiration/wash process for five times as conducted in step 4.<br>7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 25 minutes at 37° C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.<br>8. Add 50 µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.<br>9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.<br><br>Note:<br>1. Assay preparation: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at -20°C until the kit expiration date.<br>2. Samples or reagents addition: Please use the freshly prepared Calibrator. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each |

## Application Details

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| | pipetting step, without interruption. Duplication of all calibrators and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each calibrator level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.<br>3. Incubation: 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. Incubation time and temperature must be observed.<br>4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.<br>5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.<br>6. TMB Substrate is easily contaminated. Please protect it from light. |
| Calculation of Results: | Average the duplicate readings for each calibrator, control, and samples and subtract the average zero calibrator optical density. Create a calibration curve on log-log graph paper, with BNIP3 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the calibrator points and it can be determined by regression analysis. Using some plot software is also recommended. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor. |
| Restrictions: | For Research Use only |

## Handling

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| Storage: | -20 °C |
| Storage Comment: | All the reagents should be kept according to the labels on vials. The Calibrator, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20° C upon being received. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. |
| Expiry Date: | The expiry date is stated on the label. |