OriGene Technologies Inc.
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Human Nesfatin-1 ELISA Kit

Catalog Number: *EA100670*

Assay Principle

The OriGene Human NUCB2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human NUCB2 with a 96-well strip plate that is pre-coated with antibody specific for NUCB2. The detection antibody is a biotinylated antibody specific for NUCB2. The capture antibody is monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat. The kit includes Human NUCB2 protein as standards.

To measure Human NUCB2, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human NUCB2 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human NUCB2 in the sample

Overview

Product Name	*Human Nesfatin-1/NUCB2/Nucleobindin-2 ELISA Kit*
Reactive Species	*Human*
Size	*96wells/kit, with removable strips.*
Description	*Human Nesfatin-1 ELISA Kit (96 Tests). Quantitate Human NUCB2 in cell culture supernatants and serum. Sensitivity: 10pg/ml.*
Sensitivity	*<10 pg/ml*
Detection Range	*31.2 pg/ml - 2,000 pg/ml*
Storage Instructions	*Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)*
Uniprot ID	*P80303*

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Technical Details

Capture/Detection Antibodies	*The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.*
Specificity	*Natural and recombinant Human NUCB2*
Immunogen	*Expression system for standard: E.coli; Immunogen sequence: V25-L106*
Cross Reactivity	*This kit is for the detection of Human NUCB2. No significant cross-reactivity or interference between NUCB2 and its analogs was observed. This claim is limited by existing techniques; therefore, cross-reactivity may exist with untested analogs.*

Notice Before Application

Please read the following instructions before starting the experiment.

1. *To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.*
2. *Before using the Kit, spin tubes and bring down all components to the bottom of tubes.*
3. *Don't let 96-well plate dry, for dry plate will inactivate active components on plate.*
4. *Don't reuse tips and tubes to avoid cross contamination.*
5. *Avoid using the reagents from different batches together.*

Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human NUCB2 Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**	**Return unused wells to the foil pouch. Reseal along the entire edge of the zip seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Human NUCB2 Standard	**2**	**2 ng/tube**	**Discard the NUCB2 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.**
Human NUCB2 Biotinylated antibody (100x)	**1**	**100 µl**	**May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 µl**	
Sample Diluent	**1**	**30ml**	
Antibody Diluent	**1**	**12ml**	
Avidin-Biotin-Peroxidase Diluent	**1**	**12ml**	
Color Developing Reagent (TMB)	**1**	**10ml**	
Stop Solution	**1**	**10ml**	
Wash Buffer (25x)	**1**	**20 ml**	
Plate Sealers	**4**	**Piece**	

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Required Materials That Are Not Supplied

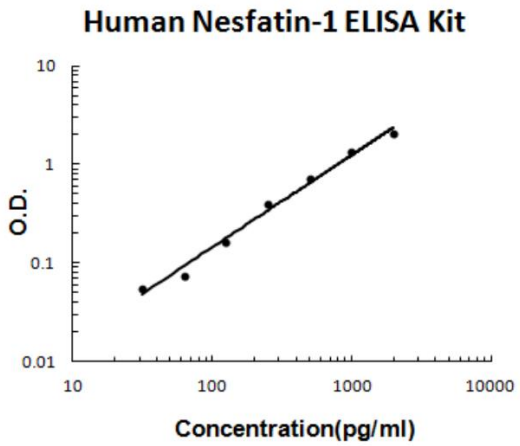
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human NUCB2 ELISA Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	31.2	62.5	125	250	500	1000	2000
O.D.	0.032	0.086	0.106	0.196	0.432	0.755	1.370	2.094

Human NUCB2 ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*

Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

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	Intra-Assay Precision			Inter-Assay Precision		
Sample	*1*	*2*	*3*	*1*	*2*	*3*
n	*16*	*16*	*16*	*24*	*24*	*24*
Mean(pg/ml)	*58*	*278*	*993*	*62*	*286*	*1045*
Standard deviation	*2.55*	*17.51*	*57.20*	*3.41*	*20.59*	*74.19*
CV(%)	*4.4*	*7.2*	*5.6*	*5.5*	*7.2*	*7.1*

Reproducibility

To assay reproducibility, three samples with different target protein concentrations were assayed using four different lots. Number of each test n = 16.

Lots	*Lot 1(pg/ml*	*Lot2 (pg/ml)*	*Lot3 (pg/ml)*	*Lot4 (pg/ml)*	*Mean (pg/ml)*	*Standard Deviations*	*CV (%)*
Sample1	*58*	*50*	*49*	*55*	*53*	*3.67*	*6.9*
Sample2	*278*	*249*	*272*	*250*	*262*	*12.93*	*4.9*
Sample3	*993*	*1004*	*914*	*846*	*939*	*64.06*	*6.8*

Preparation Before The Experiment

Item	*Preparation*
All reagents	*Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.*
Wash buffer	*Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.*
Biotinylated Anti-human SPARC antibody	*It is recommended to prepare this reagent immediately prior to use by diluting the Human NUCB2 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.*
Avidin-Biotin-Peroxidase Complex	*It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin- Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.*
Human SPARC Standard	*It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 2 ng of lyophilized Human NUCB2 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 2 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.*

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Microplate	*The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.*

Dilution of Human NUCB2 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 2,000.00 pg/ml, # 2: 1,000.00 pg/ml, # 3: 500.00 pg/ml, # 4: 250.00 pg/ml, # 5: 125.00 pg/ml, # 6: 62.50 pg/ml, # 7: 31.25 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. For standard #1, add 1000 µl of undiluted standard stock solution to tube #1.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube # 4-7.

Sample Preparation andStorage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.
Sample dilution ratios should be determined by a pilot study (run a serial dilution of samples and see which dilution ratio results in the idea O.D., near the middle of the standard range). In general, high concentration samples can be dilutioned by 1:100, mid concentration samples 1:10, low concentration samples 1:2 or neat.

Sample Type	*Procedure*
Cell culture supernatants	*Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.*
Serum	*Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.*

Sample Collection Notes

1. Boster recommends that samples are used immediately upon preparation.

2. Avoid repeated freeze/thaw cycles for all samples.

3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.

4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.

5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.

6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.

7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

8. Boster is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

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Sample Dilution

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. *Prepare all reagents and working standards as directed previously.*
2. *Remove excess microplate strips from the plate frame and seal and store them in the original packaging.*
3. *Add 100 µl of the standard, samples, or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended.*
4. *Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C).*
5. *Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
6. *Add 100 µl of the prepared 1x Biotinylated Anti-Human NUCB2 antibody to each well.*
7. *Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).*
8. *Wash the plate 3 times with the 1x wash buffer:*

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.

   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
9. *Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).*
10. *Wash the plate 5 times with the 1x wash buffer:*

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash)

    c. Repeat steps a-b 4 additional times.

    d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.

    11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)

    12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.

    13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

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Assay Protocol Notes

1. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

2. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.

3. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.

4. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.

5. Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.

6. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.

7. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.

8. Reaction Time Control: Control reaction time should be strictly followed as outlined.

9. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.

10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, 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.

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Data Analysis

To analyze using manual methods, follow the process below:

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on NUCB2

Nesfatin-1 is a naturally occurring protein molecule produced by the brains of mammals. It is responsible for regulating appetite and production of body fat. Nesfatin-1 is a metabolic polypeptide encoded in the N-terminal region of the protein precursor, Nucleobindin2 (NUCB2). Recombinant human Nesfatin-1 is a 9.7 kDa protein containing 82 amino acid residues. Originally identified as a hypothalamic neuropeptide, Nesfatin-1 is also expressed in other areas of the brain, and in pancreatic islets of Langerhans, gastric endocrine cells and adipocytes. Nesfatin-1 plays a role in hunger and energy regulation in an independent manner.