Human Sushi, nidogen and EGF-like domain-containing Protein 1 (SNED1) ELISA Kit

Cat #: orb2809933 (manual)

Size: 96 tests / 48 tests

For research use only, not for clinical diagnosis.

SNED1 Introduction

SNED1 protein (SNED1) is an extracellular matrix (ECM) protein expressed at low levels in various tissues. It contains characteristic structural features of ECM proteins, including an N-terminal NIDO domain, several calcium-binding EGF-like domains (EGF_CA), a Sushi domain, also known as a complement control protein (CCP) domain, and three fibronectin type III (FN3) domains in the C-terminal region.

Detection Principle

This kit is based on double antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotinylated detection antibodies, and HRP enzyme conjugates were added to the microwells pre-coated with human SNED1 capture antibody, incubated and washed, and then developed using substrate TMB. TMB is converted to blue under the catalysis of peroxidase (HRP) and then transformed into the final yellow under acidic conditions. The shade of colour was positively correlated with the human SNED1 in the sample. Measure the absorbance (OD value) with a microplate reader at a wavelength of 450 nm and calculate the sample concentration.

Detection Range: 0.312-20ng/mL

Sensitivity: 0.151ng/mL

Specificity: detects human SNED1 in samples with no significant cross-reactivity with its analogues.

Product Composition

Reagents	Specifications（96T）	Specifications（48T）	Storage Conditions
Pre-coated Assay Plate	8×12	8×6	2-8℃
Standard	2 tubes	1 tube	2-8℃
Universal Diluent	2×20 mL	1×20 mL	2-8℃
Biotin-antibody (100×)	120 µl	60 µl	2-8℃
Streptavidin-HRP (100×)	120 µl	60 µl	2-8℃
Wash Buffer (20×)	2×10 mL	1×10 mL	2-8℃

TMB Substrate	10 ml	5 ml	2-8°C
Stop Solution	6 ml	3 ml	2-8°C
Plate Sealer	4 pieces	4 pieces	
Manual	1 copy	1 copy	

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision pipette with disposable tips: 0.5-10uL, 5-50uL, 20-200uL, 200-1000uL
3. 37°C incubator
4. Deionized or distilled water

Precautions

1. Incubation is carried out in strict accordance with the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate reagents immediately after use.
2. Incorrect plate washing may result in inaccurate results. Make sure to suction out as much liquid as possible from the wells before adding the substrate. Do not allow the micropores to dry out during the incubation process.
3. Eliminate residual liquid and finger marks from the bottom of the plate, otherwise the OD value will be affected.
4. The substrate chromogenic solution should be colourless or very light in colour, and the substrate solution that has turned blue cannot be used.
5. Avoid cross-contamination of reagents and specimens to avoid false results.
6. Avoid direct exposure to strong light during storage and incubation.
7. No reaction reagent should come into contact with the bleaching solvent or the strong gases emitted by the bleaching solvent. Any bleaching component will destroy the biological activity of the reaction reagent in the kit.
8. Expired products cannot be used, and components with different article numbers and batch numbers should not be mixed.
9. Recombinant proteins from sources other than the kit may not match the antibody of this kit and may not be recognized.
10. If there is a possibility of disease transmission, all samples should be managed, and samples and testing devices should be handled in accordance with the prescribed procedures.

Sample Collection and Requirements

1. **The detection range of the kit is not equivalent to the concentration range of the analyte in the sample,** and it is recommended to estimate the concentration of the analyte in the sample through relevant literature before the experiment and determine the actual concentration of the sample through the pre-

experiment Condition. If the concentration of analyte in the sample is too high or too low, dilute or concentrate the sample appropriately.

2. If the sample to be tested is not among the samples listed in the manual, it is recommended to do a pre-test to verify the validity of the test.

3. **Serum:** Whole blood specimens collected in serum separators are stored at room temperature for 2 hours or 2-8°C overnight, then centrifuge at 1000×g for 20 minutes, and the supernatant can be taken or stored at -20°C or -80°C, but repeated freeze-thaw should be avoided.

4. **Plasma:** Collect specimens with EDTA or heparin as anticoagulants and centrifuge the specimen at 1000×g at 2-8°C for 15 minutes within 30 minutes of collection, take the supernatant for detection, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.

5. **Tissue homogenate:** Rinse the tissue with pre- cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate can affect the measurement), and mince the tissue after weighing. The minced tissue is compared to the corresponding volume of PBS (generally according to the weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to the needs of the experiment, and a record should be made. It is recommended to add protease inhibitors to PBS) to a glass homogenizer and grind well on ice. To further lyse the histiocytes, the homogenate can be sonicated or repeatedly freeze-thaw. Finally, the homogenate was centrifuged at 5000×g for 5~10 minutes, and the supernatant was taken for detection.

6. **Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes to detect or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.

7. **Cell lysate:** For adherent cells, gently wash with pre-cooled PBS, then digest with trypsin, and collect the cells by centrifuging at 1000×g for 5 minutes; suspended cells can be collected directly by centrifugation. Wash the collected cells with pre-cooled PBS 3 times, and resuspend 150-200μL PBS for every 1×10^6 cells (it is recommended to add a protease inhibitor in the PBS; if the content is very low, you can appropriately reduce the volume of PBS) and lyse the cells by repeated freeze-thaw cycles or sonication. Centrifuge the extracted solution at 1500×g for 10 minutes at 2-8°C and collect the supernatant for detection.

8. **Other biological specimens:** Centrifuge at 1000×g for 20 minutes and take the supernatant for detection.

9. **Appearance of the sample:** The sample should be clear and transparent, and the suspended solids should be centrifuged and removed.

10. **Sample preservation:** If the sample is tested within 1 week after collection, it can be stored at 4°C, if it cannot be tested in time, please divide it into one-time use and freeze it at -20°C (test within 1 month), or -80°C (testing within 6 months), avoid repeated freezing and thawing, haemolysis of the specimen will affect the final test result, so haemolyzed specimens should not be tested for this test.

Sample Dilution Protocol

Please estimate the concentration range of the samples in advance. If your test sample needs to be diluted, refer to the dilution protocols below.

Dilution 100 times: One-step dilution. Take 5μL of the sample and add it to 495μL of universal diluent to make a 100-fold dilution;

Dilution 1000 times: Two-step dilution. Take 5μL of the sample and add it to 95μL of universal diluent to make a 20-fold dilution, then take 5μL of the 20-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 1000 times;

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Dilution 100000 times: Three-step dilution. Take 5μL of the sample and add it to 195μL of universal diluent to make a 40-fold dilution, then take 5μL of the 40-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, and finally take 5μL of the 2000-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 100000 times;

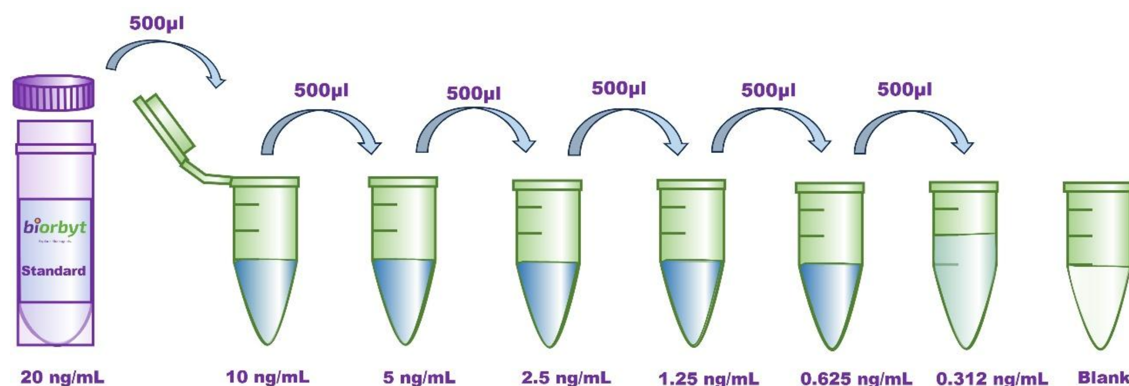
For each dilution step, the volume taken should be no less than 3μL, and the dilution factor should not exceed 100 times. Each dilution step must be mixed thoroughly to avoid foaming.

Reagent Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate to room temperature.

2. **Standard gradient working solution:** Add 1mL of universal dilution to the lyophilized standard, let it stand for 15 minutes, let it completely dissolve, and then mix gently (at a concentration of 20 ng/mL), then dilute at the following concentrations: 20ng/mL, 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0ng/mL.

Doubling dilution method: take 7 EP tubes, add 500μL of general dilution solution to each tube, and draw 500μL from 20 ng/mL standard working solution to the first EP tube to mix well to prepare 10 ng/mL standard working solution, and then pipette and mix well according to this step. The last tube is used directly as a blank well, and there is no need to draw liquid from the penultimate tube, as shown in the figure below.



3. **Preparation of biotinylated antibody detection solution:** 15 minutes before use, centrifuge the concentrated biotinylated antibody at 1000×g for 1 minute, and dilute the 100× concentrated biotinylated antibody into a 1× working concentration (10 μL concentrated + 990 μL universal diluent) in a universal diluent, and use it on the same day.

4. **Preparation of enzyme conjugate working solution:** 15 minutes before use, centrifuge 100× concentrated streptavidin-HRP at 1000×g for 1 minute, and dilute 100× concentrated streptavidin-HRP to 1× working concentration in a universal diluent (10 μL concentrated + 990 μL universal diluent) for use on the same day.

5. **Preparation of 1× washing solution:** take 10ml of 20× washing solution to 190ml of distilled water (the concentrated washing solution taken out of the refrigerator may have crystallization, which is a normal phenomenon, it can be placed at room temperature, shake evenly, and then configure after the crystallization is completely dissolved).

Operation Steps

1. Remove the desired slats from the foil pouch after 10 minutes of room temperature equilibration and place the remaining slats back at 4°C with a ziplock bag.

2. Loading: Add 100 µl of samples or standards of different concentrations to the corresponding wells and add 100 µL of universal diluent to the blank wells. Incubate for 1 h at 37°C after covering with the plate sealer. (Suggestion: Samples to be tested are diluted by a minimum of 1-fold with a universal diluent before being added to the microplate for testing to reduce the impact of matrix effects on the test results, and the sample concentration should be multiplied by the corresponding dilution factor when calculating the sample concentration. All samples and standards to be tested are recommended to be double-well).

3. Add biotin-antibody: Remove the plate and discard the liquid without washing. 100µL of biotin-antibody working solution was directly added to each well, and incubated at 37°C for 1 hour after covering with a plate sealer.

4. Wash the plate: Discard the liquid, add 300µL of 1x wash solution to each well, let it stand for 1 minute, shake off the wash solution, pat dry on absorbent paper, and repeat the wash 3 times. (You can also use a plate washer to wash the plate.)

5. Add streptavidin-HRP working solution: Add 100µL of streptavidin-HRP working solution to each well, cover the plate and incubate at 37°C for 30 minutes.

6. Wash the plate: Discard the liquid and wash the plate 5 times according to the washing method in step 4.

7. Add TMB Substrate: 90 µL of TMB Substrate was added to each well, covered with a plate sealer, and incubated at 37°C for 15 minutes in the dark.

8. Add stop solution: Remove the microplate, add 50 µL of stop solution directly to each well, and immediately measure the OD value of each well at a wavelength of 450 nm.

Result Judgment

1. Calculate the average OD value of the standard and sample replicate wells and subtract the OD value of the blank well as the correction value. Using the concentration as the abscissa and the OD value as the ordinate, the standard curve of the four-parameter logistic function is drawn on a double-logarithmic coordinate paper (the value of the blank group is removed when plotting).

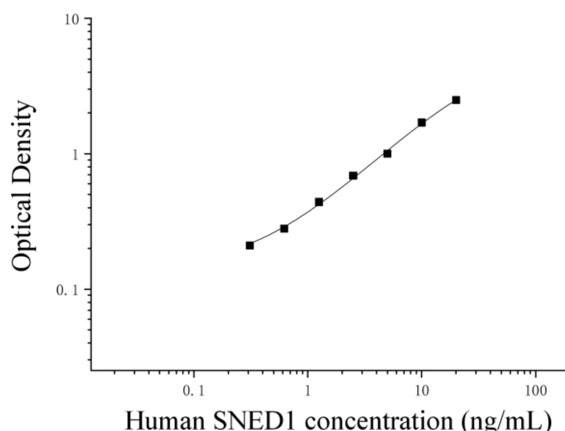
2. If the OD value of the sample is higher than the upper limit of the standard curve, it should be diluted appropriately and remeasured and multiplied by the corresponding dilution factor when calculating the sample concentration.

The following data and standard curves are for reference only, and experimenters need to establish standard curves according to their own experiments.

Concentration (ng/mL)	20	10	5	2.5	1.25	0.625	0.312	0
OD value	2.65	1.86	1.16	0.85	0.60	0.44	0.37	0.16
Adjusted OD value	2.49	1.70	1.00	0.69	0.44	0.28	0.21	-

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



Note: This figure is for reference only, and the specimen content should be calculated using the standard curve drawn for the same test standard.

Kit Performance

1. Repeatability: The coefficient of variation within the plate is less than 10%, and the coefficient of variation between plates is less than 10%.

2. Recovery: Recovery was calculated by adding 3 different concentration levels of human SNED1 to selected healthy human serum and plasma.

Sample type	Range	Average recovery
Serum （n=8)	84-113	103
Plasma (n=8)	82-114	99

3. Linear dilution: Linearity was assessed by adding high concentrations of human SNED1 to 4 selected healthy human serum and plasma, respectively, and diluting within the standard curve kinetics.

Dilution range	Recovery （%）	Serum	Plasma
1：2	Range （%）	85-104	92-105
	Average recovery （%）	96	99
1：4	Range （%）	82-110	84-114
	Average recovery （%）	95	99

Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions

After stopping the reaction, the entire plate shows a uniform yellow or light color, or the standard curve is linear but the background signal is excessively high.	Yellowing of the entire plate may be caused by incorrect addition of reagents.	Before starting the experiment, verify the components and lot numbers of all reagents to ensure they belong to the same kit. Reagents from different kits or different lot numbers must not be mixed.
	The ELISA plate was not washed sufficiently.	Ensure that the same volume of Wash Solution is added to each microwell during the washing process. After washing, firmly tap the ELISA plate on absorbent paper to remove any residual buffer.
	Incubation time was too long.	Strictly follow the procedures outlined in the manual.
	Streptavidin-HRP contaminated the pipette tip or TMB container, or the positive control contaminated the pre-coated microplate.	Replace pipette tips when dispensing different reagents. Use separate containers when preparing different reagent components, and always use a pipette during handling.
	The concentration of Biotinylated Antibody or Streptavidin-HRP was too high.	Verify that concentration calculations are correct, or perform further dilution if necessary.
	The substrate was exposed to light or contaminated prior to use.	Store reagents in the dark at all times prior to substrate addition.
	Color development time was too long.	Strictly follow the procedures outlined in the manual.
	An incorrect filter was used when reading the absorbance value.	When TMB is used as the substrate, measure absorbance at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless, and the positive control is not clearly detectable. s	Components from different reagent sets were mixed.	Carefully read reagent labels when preparing or using them.
	During plate washing or sample/enzyme addition, the enzyme label was contaminated or inactivated, resulting in loss of its ability to catalyze color development.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), and ensure that the container used to prepare the Wash Solution has been thoroughly cleaned.
	A reagent or procedural step was omitted.	Review the manual carefully and strictly follow the operating procedures.

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard appears normal, but the sample color is weak.	The sample contains NaN_3 preservative, which inhibits the enzyme reaction.	Samples must not contain NaN_3.
	The sample being tested may not contain strongly positive material, so the result may be normal.	If there is any doubt about the results, repeat the assay.
The visual result appears normal, but the microplate reader values are low.	An incorrect filter was used for absorbance measurement.	When TMB is used as the substrate, absorbance should be measured at 450 nm.
All wells, including Standards and Samples, show weak color development.	Insufficient incubation time.	Ensure accurate timing using a timer.
	Inadequate color development.	Typically 15–30 minutes.
	Excessive washing, or the dilution ratio of the concentrated Wash Buffer does not meet requirements.	Minimize the impact of washing by diluting the concentrated Wash Buffer and setting the washing time according to the manual. Accurately record the number of washes and the volume used.
	Poor quality distilled water.	The prepared Wash Buffer should be tested to confirm that the pH is neutral.
	During plate washing or sample addition, the enzyme label was contaminated or inactivated, resulting in loss of catalytic activity.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), that the container used to prepare the Wash Buffer has been thoroughly cleaned, and that the purified water used meets the required standards and is not contaminated.
	The kit has expired or was improperly stored.	Use the kit within its expiration date and store it according to the conditions recommended in the manual to avoid contamination.
	Reagents and samples were not equilibrated to room temperature before use.	Allow all reagents and samples to equilibrate at room temperature for approximately 30 minutes before use.
	Insufficient pipetting volume, overly rapid dispensing, excessive liquid remaining on the inner wall of the tip, or unclean tip walls.	Calibrate the pipette properly. Ensure tips are compatible and fit securely, pipette at an appropriate speed, and fully dispense the liquid. Tips should have clean inner walls and be used only once
Poor repeatability.	Incubation temperature was not properly controlled.	Maintain a constant incubation temperature and avoid localized temperature extremes.
	Excess liquid remained on the inner wall of the wells during	When adding liquids, dispense along the lower inner wall of the wells without

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27713. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

	liquid addition.	touching the bottom.
	Reuse of consumables.	Replace pipette tips when drawing different reagents, and use separate containers when preparing different reagent components.
	The bottom of the microwell is scratched or contaminated.	Handle the plate carefully. Avoid touching the well bottoms and clean the bottom of the microplate to remove dirt or fingerprints.
		Perform technical replicates of the same sample three times, ensuring that at least two values are comparable.
	Cross-contamination during sample addition.	Minimize the risk of cross-contamination during sample addition.
The color development across the plate is uneven and irregular.	Cross-contamination during manual plate washing.	When washing plates manually, discard the first three washes immediately, then allow soaking during subsequent washes to reduce cross-contamination.
	Cross-contamination during plate tapping.	Use appropriate absorbent paper when tapping the plate. Avoid introducing foreign material into the wells, and avoid tapping in the same position repeatedly to reduce cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color development of the plate is uneven and irregular	The liquid dispensing head of the plate washer is clogged, leading to improper liquid dispensing or excessive residual liquid after aspiration, which results in uneven and irregular color development across the plate.	Unclog the liquid dispensing head to ensure that each well is properly filled with wash solution during plate washing and that minimal residual liquid remains after aspiration.
	Incomplete centrifugation of the sample, resulting in coagulation within the reaction wells or interference from sediment or residual cellular components.	Serum and plasma samples should be fully centrifuged at 3000 rpm for more than 6 minutes.
	The sample was stored for an excessively long period, leading to contamination.	Samples should be kept fresh or stored at low temperatures to prevent contamination.
	Incorrect preparation of the Wash Solution or direct use of the concentrated Wash Solution.	Prepare all reagents strictly according to the manual.