HUMAN NIDOGEN-2 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN NIDOGEN-2 CONCENTRATIONS IN SERUM AND PLASMA.

PURCHASE INFORMATION:

ELISA NAME	HUMAN NIDOGEN-2 ELISA
Catalog No.	SK00480-01
Formulation	96 T
Standard Range	3.12-200 ng/ml
Sensitivity	50 pg/mL
Sample Volume	100 µl per well
Sample Type	Serum, plasma
Specificity	Human Nidogen-2
Sample Dilution	200
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

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FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Human Nidogen-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human Nidogen-2 in serum. It contains recombinant human Nidogen-2 and antibodies raised against this protein. It has been shown to accurately quantitie human Nidogen-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Nidogen-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Nidogen-2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human Nidogen-2 is added to the wells. Following a wash to remove any unbound antibody reagent. After washing away any unbound antibody, a Streptavidin HRP conjugate is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Nidogen-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer 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.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Nidogen-2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human Nidogen-2.	480-01-01	1 plate
Nidogen-2 Standard – 200 ng/vial of human Nidogen-2 in a buffered protein base with preservatives; lyophilized.	480-01-02	1 vial
Detection Antibody Concentrated – 105 µL / vial, 100-fold concentrated of biotinylated Antiobdy against human Nidogen-2 with preservatives;	480-01-03	1 vial
Positive Control- one of human Nidogen-2, lyophilized	480-01-04	1 vial
Streptavidin HRP Conjugate- 130 µL of 100-fold concentrated Streptavidin HRP Conjugate	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB08	1 vial
HRP Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	DB01	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution -11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: Reconstituted Detection Antibody and Standard Stock may be stored for up to 1 month at -70°C. Streptavidin HRP

conjugate 100 fold concentrated should be stored at 2 - 8° C .

Microplate Wells: Return unused wells to the plastic zip bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

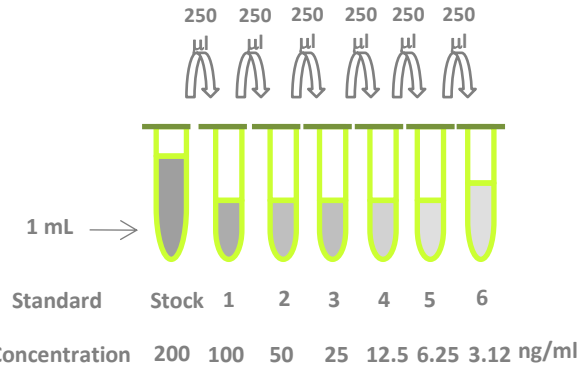
REAGENT PREPARATION

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

Nidogen-2 Standard - Refer to vial label for reconstitution volume. Reconstitute the Nidogen-2 Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	200 pg/ml
# 1	250 µl of stock	250 µl	100 pg/ml
# 2	250 µl of 1	250 µl	50 pg/ml
# 3	250 µl of 2	250 µl	25 pg/ml
# 4	250 µl of 3	250 µl	12.5 pg/ml
# 5	250 µl of 4	250 µl	6.25 pg/ml
# 6	250 µl of 5	250 µl	3.125 pg/ml



Detection Antibody – Reconstitute the Detection Antibody concentrated with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin HRP Conjugate –Pipette 11.88 mL of the appropriate HRP Diluent Solution into the 15 ml centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution.

Positive Control –Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare working solution.

ASSAY PROCEDURE

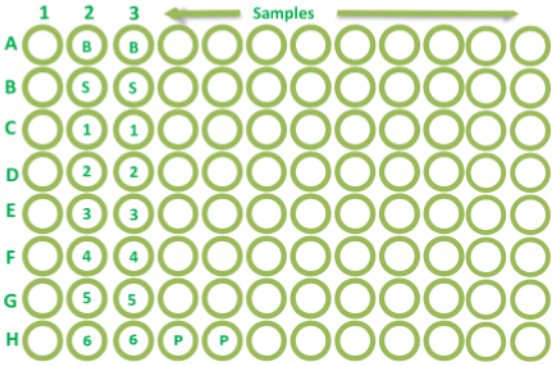
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (A2, A3).
4. Add 100 µL of Standard (from B2, B3 to H2, H3,), sample, or control per well (H4, H5). Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300µL) using a squirt bottle, 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin HRP working solution to each well. Incubate for 45 minutes at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 4-7 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Nidogen-2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. 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.



CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Nidogen-2.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Nidogen-2 was 50 pg/mL.

TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.108)
3.125	0.046
6.25	0.094
12.5	0.178
25	0.344
50	0.764
100	1.446
200	2.361

SPECIFICITY

PROTEINS	CROSS-REACTIVITY(%)
Human Nidogen-2	100
Human Periostin	0
Human Mesothelin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Streptavidin HRP working solution to each well. Incubate 45 minutes in a paper box (Protect from light) on the plate shaker at RT
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Substrate to each well. Incubate 4-7 min on the bench top. Protect from light.
↓
Add 100 µl of Stop Solution to each well. Read 450nm within 15 min