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**INSTRUCTION FOR USE:  
Human Secretoneurin ELISA**

Product code: 47-11

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

**1. PRODUCT DESCRIPTION**

**1.1 INTENDED USE**

The Human Secretoneurin ELISA is designed to detect and quantify the level of human secretoneurin (SN) in serum and plasma. For research use only. Not for use in diagnostic procedures.

**1.2 PRINCIPLE OF THE ASSAY**

The Human Secretoneurin ELISA is a sandwich Enzyme-linked Immunoassay (ELISA). Two secretoneurin-specific sheep monoclonal antibodies (MABs) are used in the assay. One MAB is biotinylated and the second is HRP-conjugated. The biotinylated antibody is added to the wells of a streptavidin coated microtiter plate. After washing, diluted calibrators, controls and samples are added, and secretoneurin present is bound to the immobilized antibody. After incubation of samples the wells are washed to remove unbound sample material, and the HRP-conjugated antibody is added. After incubation and removal of unbound conjugate by washing, the TMB substrate is added. The blue color developed is directly proportional to the amount of secretoneurin present in the calibrators, controls, and samples. The stop solution changes the color from blue to yellow, and the intensity of the color is measured in a microtiter plate reader.

**1.3 KIT CONTENTS**

Component	Quantity	Cap color	Reagent composition
SN microtiter plate	1 pcs., 96 well		Streptavidin coated microtiter plate
SN Calibrators	6 vials (A-F) x 100 µL	White	Secretoneurin peptide. Phosphate buffer with detergents. NaN <sub>3</sub> (0.09 %)
SN Controls	2 vials (Low-High) x 100 µL	Red	
SN 100x Biotinylated Ab	1 vial x 175 µL	Blue	Biotinylated anti-secretoneurin antibody. TRIS-HCl buffer with BSA. NaN <sub>3</sub> (0.05 %)
SN 100x HRP-Conjugate	1 vial x 175 µL	Green	HRP-conjugated anti-secretoneurin antibody. TRIS-HCl buffer
SN Conjugate Diluent	1 vial x 15 mL	Yellow	TRIS-HCl buffer
SN Assay Buffer	1 vial x 50 mL	White	TRIS-HCl buffer with BSA and detergents. NaN <sub>3</sub> (0.05 %). Red dye
SN 25x Wash Solution	1 vial x 100 mL	White	TRIS-HCl buffer with detergents and preservative
SN Substrate	1 vial x 20 mL	Black	TMB substrate
SN Stop Solution	1 vial x 15 mL	Red	Sulfuric acid 0.5 M

**1.4 MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT**

- Distilled or deionized water
- Microtiter plate reader for measurement of absorbance at, or near, 450 nm
- Microtiter plate washer – automated or manual
- Microtiter plate shaker
- Calibrated, adjustable precision pipettes with disposable tips
- Glass, or plastic tubes for preparation of required dilutions
- Lid/cover for microtiter plate
- Centrifuge

## 2. WARNINGS AND PRECAUTIONS

- This kit is for **Research Use Only**
- Do not pipette by mouth
- Do not eat, drink, smoke or apply cosmetics where specimens and reagents are handled
- Use disposable gloves to avoid contact with specimens and reagents
- Waste should be disposed of in accordance with local, national, or regional regulations. Safety Data Sheets (SDS) are available upon request
- Sodium azide (NaN<sub>3</sub>), even in low concentrations less than 0.1 %, may react with lead or copper plumbing to form potentially explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up
- Sulfuric acid is irritating to eyes and skin. Wear protective gloves/protective clothing/eye protection when handling sulfuric acid. Flush with water if contact occurs

## 3. SAMPLE COLLECTION, PREPARATION AND STORAGE

Serum, EDTA plasma, and Li-heparin plasma can be used for testing secretoneurin with the Human Secretoneurin ELISA Assay. Underfilling of EDTA and/or Li-Heparin tubes have not been found to affect the secretoneurin result.

It is Important to note that secretoneurin in serum and plasma degrades and is only stable at room temperature for four hours. Samples should not be stored at room temperature. Samples are found to be stable for 3-4 days refrigerated (2-8°C / 36-46°F).

For long term storage it is recommended to keep samples at - 80°C / - 112°F.

The number of freeze/thaw cycles for K2-EDTA plasma, Li-Heparin plasma, and serum samples should be kept as few as possible to avoid any degradation of secretoneurin. It is recommended to aliquot samples before storage at - 80°C / - 112°F.

## 4. REAGENT STORAGE

Kits shall be stored at 2-8°C / 36-46°F.

Do not use the kit beyond the expiry date stated on the label of the outer container.

Once opened, kits shall be used the same day and not stored for later use.

Working solutions of samples, calibrators, controls, biotinylated antibody, and HRP-conjugate are stable for six hours, and the diluted wash solution is stable for eight hours at room temperature.

## 5. ASSAY PROCEDURE

### Before you begin

- Thoroughly review this procedure
- **Important!** Reagents are lot specific. Do not mix or interchange reagents of different lots.
- Once opened the kit must be used within one working day.
- The SN substrate is light sensitive and stored in a dark, light protected bottle. Do not expose the substrate to direct light.

Allow reagents, samples, and microtiter plate to reach room temperature before use.

Centrifuge vials before opening to avoid liquid being trapped in the lid.

### Preparation of samples and reagents:

1. Wash Solution (1x): Dilute 40 mL of SN 25x Wash Solution to 1000 mL in distilled/deionized water
2. Dilute SN 100x Biotinylated Antibody 1:100 in SN Assay Buffer
3. Dilute SN Calibrators (A-F), SN Controls (Low-High) and samples 1:10 in SN Assay Buffer. Mix well before and after dilution. Do not dilute directly in the microtiter plate well
4. Dilute the SN 100x HRP-conjugate 1:100 in SN Conjugate Diluent
5. Samples with a concentration above the measuring range shall be diluted in assay buffer and re-run

### ELISA test procedure.

Total assay time is approx. three hours.

The assay should be performed at a temperature between 15–26°C / 59–79°F .

After each wash, proceed immediately to next assay step.

**Important! Perform a calibration curve with each run.**

1. Add 100µL diluted SN Biotinylated Antibody to each well and incubate the microtiter plate at room temperature on a shaker for 30 - 120 minutes
2. Wash the microtiter plate three times using 350 µL (1x) Wash Solution per well
3. Add 100µL of diluted SN Calibrators/SN Controls/samples in duplicate to appropriate wells and incubate the plate at room temperature on a shaker for 45 – 90 minutes
4. Wash the microtiter plate three times using 350 µL (1x) Wash Solution per well
5. Add 100µL of diluted HRP-Conjugate to each well and incubate the microtiter plate at room temperature on a shaker for 45 - 90 minutes
6. Wash the microtiter plate six times using 350 µL (1x) Wash Solution per well
7. Add 150 µL SN Substrate to each well and incubate the microtiter plate for 10 minutes (+/- 30 sec). No shaking. Cover the microtiter plate to protect from light or incubate in a dark place. A blue color develops in the wells **Important:** Make sure that the SN Substrate does not get in contact with aluminium foil or other metals
8. Add 50 µL SN Stop Solution to each well and mix. The solution changes from blue to yellow
9. Read the microtiter plate at 450 nm within 20 minutes

5.1 CALCULATION OF SAMPLE CONCENTRATIONS

The calibration curve is obtained by plotting the absorbance readings of the calibrators against the corresponding calibrator concentrations. Use least square linear regression for calculating sample secretoneurin concentrations. Check that the calibration curve is linear.

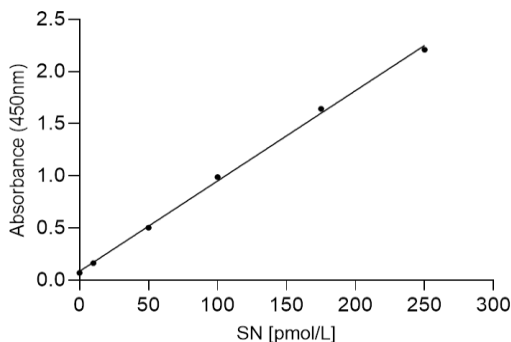


Figure 1: Example, Calibration Curve.

Measuring range: 10 - 250 pmol/L

5.2 ASSAY CALIBRATION AND QUALITY CONTROL

The calibrators included in the Human Secretoneurin ELISA kit are lot specific. The concentrations are stated in the certificate of analysis included with each kit.

The Human Secretoneurin ELISA kit includes two controls: control low and control high. The reference values of the controls are stated in the certificate of analysis provided with each kit.

6. ANALYTICAL PERFORMANCE

6.1 SINGLE SITE PRECISION

Repeatability and within-device precision were established according to the CLSI guideline EP05-A. Three serum samples (S1, S2, S3) with low, medium, and high concentrations of secretoneurin, as well as low and high controls were tested in this study. Medium and high samples were spiked to reach the desired secretoneurin concentration. All samples were analysed twice a day in two replicates for 20 days, by one operator.

Table 4: Results from single site precision study (n=80)

Samples	Mean value pmol/L	Repeatability (within-run precision)		Within Device precision (total precision)	
		SD	%CV	SD	%CV
S1	32.6	0.9	2.7	2.8	8.7
S2	71.5	2.6	3.6	4.8	6.7
S3	155.4	4.3	2.8	9.1	5.9
Low Control	44.0	2.0	4.6	3.3	7.6
High Control	176.6	4.8	2.7	9.3	5.3

6.2 LEVEL OF DETERMINATION (LoD)

LoD was established according to the CLSI guideline EP17. The LoD for the CardiNor Secretoneurin ELISA Assay is 5.1 pmol/L, based on 192 determinations, with 96 blank and 96 low level replicates, and a Level of Blank (LoB) of 2.7 pmol/L. The proportions of false positives ( $\alpha$ ) are less than 5% and false negatives ( $\beta$ ) less than 5%.

6.3 LEVEL OF QUANTIFICATION (LoQ)

LoQ was established according to the CLSI guideline EP17. The LoQ for the CardiNor Secretoneurin ELISA Assay is 7.6 pmol/L, based on 96 determinations performed by two operators to include operator to operator variation.

6.4 LINEARITY

Linearity was established according to the CLSI guideline EP06-A. The CardiNor Secretoneurin ELISA Assay is linear from 11.8 to 299.2 pmol/L with a %CV of 7.6 for secretoneurin above 15 pmol/L, and a %CV of 13.0 for secretoneurin below 15 pmol/L.

6.5 HOOK EFFECT

No falsely low secretoneurin results were observed for serum samples up to 5000 pmol/L tested with the CardiNor Secretoneurin ELISA Assay.

7. LIMITATIONS

Once opened, the Human Secretoneurin ELISA test kit must be used the same day. Remaining unused wells of the microtiter plate cannot be used over several days.