CARDINOR CE

INSTRUCTION FOR USE

CardiNor Secretoneurin ELISA Assay

REF 100-01

For *In Vitro* Diagnostic Use

1. PRODUCT DESCRIPTION

1.1 INTENDED USE

The CardiNor Secretoneurin ELISA Assay is an in vitro diagnostic test designed to detect and quantify the level of human secretoneurin (SN) in serum and plasma. It is indicated to be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with heart failure.
The test is intended to be used by professionals.

1.2 SECRETONEURIN AS BIOMARKER

Secretoneurin (SN) is a 33-amino acid peptide and the biological active fragment of secretogranin II[1], a protein in the granin family[2]. SN is produced by neuroendocrine- and heart muscle cells and is detectable in the blood stream[3].
Circulating secretoneurin concentrations are increased in patients with acute heart failure in proportion to disease severity in patients with arrhythmia-induced cardiac arrest[1], after coronary artery bypass grafting[4], and in critically ill patients with severe infections[3]. Secretoneurin is a strong predictor of mortality in patients with acute heart failure[1], in acute respiratory failure patients with CVD[5], in patients with severe sepsis[3,6], and in patients with acute coronary syndrom[7].

1.3 PRINCIPLE OF THE ASSAY

CardiNor's method for measurement of secretoneurin is an ELISA (Enzyme-linked immunosorbent assay) based on two secretoneurin-specific monoclonal antibodies (MABs) from sheep. 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 wash the TMB substrate is added. The blue colour developed is directly proportional to the amount of secretoneurin present in the calibrators, controls, and samples. The stop solution changes the colour from blue to yellow, and the intensity of the colour is measured in a microtiter plate reader.

1.4 KIT CONTENTS AND PREPARATION OF REAGENTS

Table 1: Kit composition and preparation of reagents.

REF / Component	Quantity	Cap colour	Reagent composition	Reagent preparation
100-02 SN microtiter plate	1 pcs., 96 well		Streptavidin coated microtiter plate	
100-05 SN Calibrators	6 vials (A-F) x 100 µL	White	Secretoneurin. Phosphate buffer with detergents. NaN_3 (0.09 %)	Dilute SN Calibrators (A-F) 1:10 in SN Assay Buffer (white cap)
100-06 SN Controls	2 vials (Low-High) x 100 µL	Red		Dilute SN Controls (low-high) 1:10 in SN Assay Buffer (white cap)
100-03 SN 100x Biotinylated antibody	1 vial x 175 µL	Blue	Biotinylated anti-secretoneurin antibody. TRIS-HCl buffer with BSA. NaN_3 (0.05 %)	Dilute SN 100x Biotinylated Antibody 1:100 in SN Assay Buffer (white cap)
100-04 SN 100x HRP-Conjugate	1 vial x 175 µL	Green	HRP-conjugated anti-secretoneurin antibody. TRIS-HCl buffer	Dilute SN 100x HRP-conjugate 1:100 in SN Conjugate Diluent (yellow cap)
100-10 SN Conjugate Diluent	1 vial x 15 mL	Yellow	TRIS-HCl buffer	
100-07 SN Assay Buffer	1 vial x 50 mL	White	TRIS-HCl buffer with BSA and detergents. NaN_3 (0.05 %). Red dye	
100-08 SN 25x Wash Solution	1 vial x 100 mL	White	TRIS-HCl buffer with detergents and preservative	Dilute 40 mL SN 25x Wash Solution to 1000 mL of distilled or deionized water (1x Wash solution)
100-09 SN Substrate	1 vial x 20 mL	Black	TMB substrate	
100-11 SN Stop Solution	1 vial x 15 mL	Red	Sulfuric acid 0.5 M	

1.5 MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

- Distilled or deionised water
- Microtiter plate reader for measurement of absorbance at 450 nm
- Microtiter plate washer – automated or manual
- Microtiter plate shaker
- Precision pipettes with disposable tips
- Tubes for preparing diluted samples
- Lid/cover for microtiter plate
- Centrifuge

Example Version

2. WARNINGS AND PRECAUTIONS

- This kit is for *in vitro* diagnostic 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, regional or national regulations. Safety Data Sheets (SDS) are available upon request
- Sodium azide (NaN_3), 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 CardiNor Secretoneurin ELISA Assay.

K2-EDTA, Li-Heparin, and serum samples were drawn from 116 patients. The secretoneurin concentration ranged from 15 – 160 pmol/L. No significant difference between serum, EDTA or Li-heparin plasma was found for secretoneurin.

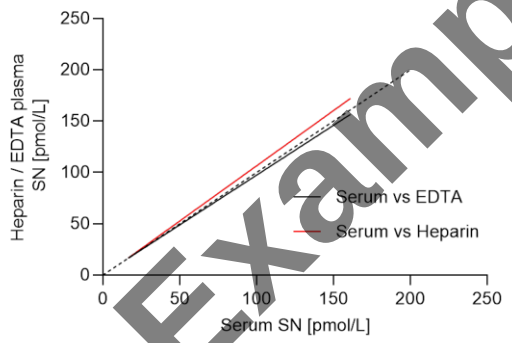

Figure 1: Comparison of serum, EDTA and Li-heparin plasma for measuring secretoneurin with the CardiNor Secretoneurin ELISA assay.

Underfilling of EDTA and/or Li-Heparin tubes did not affect the secretoneurin result.

Storage conditions for K2-EDTA plasma, Li-Heparin plasma, and serum samples for three different temperatures are shown in table 2.
Important: When stored at room temperature the concentration of secretoneurin in serum and plasma drops rapidly and is only stable for four hours. Samples should not be stored at room temperature.

The number of freeze/thaw cycles for K2-EDTA plasma, Li-Heparin plasma, and serum samples should not exceed the numbers shown in table 3.

Table 2: Sample storage.

Sample type	21 ± 2°C	2 – 8°C	- 80°C*
Serum	4 hours	3 days	12 months
K2-EDTA plasma	4 hours	4 days	12 months
Li-Heparin plasma	4 hours	4 days	12 months

*Preliminary results, ongoing study

Table 3: Maximum freeze/thaw cycles of samples.

Sample type	Maximum freeze / thaw cycles
Serum	10
K2-EDTA plasma	2
Li-Heparin plasma	3

4. REAGENT STORAGE

Kits shall be stored at 2-8 °C.
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. The diluted wash solution is stable for eight hours at room temperature.

5. ASSAY 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.
Thoroughly review this procedure before you begin.

Allow reagents, samples, and microtiter plate to reach room temperature before use.
Centrifuge vials before opening to avoid liquid being trapped in the lid.
For best analytical result, use the same pipetting equipment for calibrators, controls, and samples.

5a. 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 patient 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. Patient samples with an SN-concentration above the measuring range shall be diluted in assay buffer and re-run

Example Version

5b. ELISA test procedure.
Total assay time is approx. three hours.
The assay should be performed at a temperature between 15°C – 26°C.
After each wash, proceed immediately to next assay step.
Important! Perform a calibration curve with each run.

1. Add 100 µL diluted SN Biotinylated Antibody to each well and incubate the microtiter plate at room temperature on a shaker for minimum 30 minutes[a]
2. Wash the microtiter plate three times using 350 µL (1x) Wash Solution per well
3. Add 100 µL of diluted SN Calibrators/SN Controls/patient samples in duplicate to appropriate wells and incubate the plate at room temperature on a shaker for minimum 45 minutes[b]
4. Wash the microtiter plate three times using 350 µL (1x) Wash Solution per well
5. Add 100 µL of diluted HRP-Conjugate to each well and incubate the microtiter plate at room temperature on a shaker, for minimum 45 minutes[c]
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 colour 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

[a]The incubation time for the diluted biotinylated antibody can be between 30 – 120 minutes.
[b]The incubation time for the diluted SN Calibrators/SN Controls/patient samples can be between 45 – 90 minutes.
[c]The incubation time for the diluted HRP-Conjugate can be between 45 – 90 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. Consider excluding deviating measurements or perform a re-run.

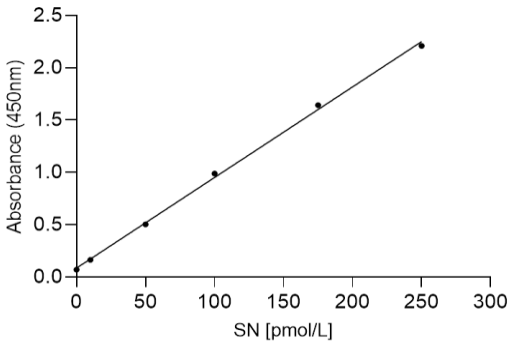

Figure 2: Example, Calibration Curve.

Measuring range: 10 - 250 pmol/L

5.2 ASSAY CALIBRATION AND QUALITY CONTROL
The concentration of the calibrators included in the CardiNor Secretoneurin ELISA kit is traceable to a set of primary standards based on crystalline secretoneurin.
The calibrator values are lot specific. Values are stated in the certificate of analysis included with each kit.

The CardiNor 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. Should the result for one or both controls be out of range, it is recommended to consider the whole run as invalid.

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 Device 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 LOT-TO-LOT PRECISION
Between-lot precision and reproducibility 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 with secretoneurin to reach the desired concentration. All samples were analysed once a day in five replicates for five days, on three reagent lots, by one operator.

Table 5: Results from Lot-to-Lot precision study (n=75, Lots=3).

Samples	Mean value pmol/L	Between Lot Precision		Between Lot Reproducibility	
		SD	%CV	SD	%CV
S1	34.4	3.1	8.9	4.2	12.1
S2	79.0	6.0	7.6	10.9	13.8
S3	165.0	10.5	6.4	16.9	10.3
Low Control	45.0	12.8	6.6	5.6	12.5
High Control	193.0	4.5	10.1	16.8	8.7

Example Version

6.3 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. The proportions of false positives (α) are less than 5% and false negatives (β) less than 5%.

6.4 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.

6.5 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.

6.6 HOOK EFFECT

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

6.7 EXOGENOUS INTERFERENCE

Exogenous interference was established according to the CLSI guideline EP07.

Serum samples have been spiked to simulate the influence of the substances listed in table 6.

The investigation showed no interference with the CardiNor Secretoneurin ELISA Assay of any of the substances and concentrations listed in table 6.

Table 6: Results from the exogenous interference study.

	Concentration tested	
Substance	**µmol/L**	**mg/dL**
Acetylsalicylic acid	170	3.0
Amiodarone	65	4.2
Amlodipine	0.18	0.0075
Atenolol	34	0.9
Biotin	14.4	0.351
Clopidogrel	1100	36
Digoxin	0.050	0.0039
Enalapril	2.2	0.082
Furosemide	48	1.6
Hydrochlorothiazide	3.80	0.113
Lisinopril	0.607	0.0246
L-Thyroxine	0.552	0.0429
Metoprolol	5.6	0.15
Quinidine	46.2	1.50
Sacubitril	22.2	0.915
Simvastatin	4.01	0.168
Valsartan	26.9	1.17
Verapamil	3.5	0.16

6.8 ENDOGENOUS INTERFERENCE

Endogenous interference was established according to the CLSI guideline EP07.

Serum samples were spiked to simulate the influence of the substances listed in table 7. The investigation showed no interference with the CardiNor Secretoneurin ELISA Assay at the concentrations tested.

Table 7: Results from the endogenous interference study.

	Concentration tested	
Substance	**mmol/L**	**mg/dL**
Bilirubin	0.7	40
Cholesterol	10	400
Haemoglobin	–	1000
Total Protein	–	15000
Triglycerides	17	1500

7. LIMITATIONS

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

8. CLINICAL PERFORMANCE

8.1 PROGNOSTIC INFORMATION

Circulating secretoneurin concentrations provide prognostic information in patients with acute and chronic heart failure[8]. Baseline secretoneurin was strongly associated with all-cause mortality in a cohort of 496 patients with ICD (Implanted cardioverter defibrillator), included in the SMASH 1 study[9] (p-value of < 0.001, see table 8).

Table 8: SMASH 1 clinical study, 28 of 496 patients with ICD died between baseline and 12 months secretoneurin (SN) measurement.

	Quartile 1 **SN < 38 pM N=124**	**Quartile 2** **SN 38-47 pM N= 125**	**Quartile 3** **SN 48-57 pM N=123**	**Quartile 4** **SN > 57 pM N=124**
One-year all-cause mortality, # of deaths	0	2	13	13

8.2 BIOLOGICAL VARIATION OF SECRETONEURIN

In a multi-centre study[10] with healthy volunteers, samples were obtained from 16 women and 14 men once a week for 10 weeks and analysed in duplicate using the CardiNor Secretoneurin ELISA Assay. Participants' median age was 36 years. Within and between subject variation (CV_I and CV_G, respectively), reference change values (RCV) and the index of individuality (II) for secretoneurin were calculated. Values for non-fasting p-glucose, eGFR, cTnT and NT-proBNP were within the normal range. No gender differences were present.

The biological variation of secretoneurin in healthy individuals is presented in table 9.

Example Version

Table 9. Biological variation of secretoneurin in healthy individuals.

	Women, n=16	Men, n=14
Median secretoneurin pmol/L	38 (p < 0.001)	33 (p < 0.001)
	Both genders	
Within subject variation (CV_I) %	9.8 (CI 8.7 to 11.0)	
Between subject variation (CV_G) %	20.0 (CI 15.4 to 28.0)	
Pos. reference change value (RCV_{pos}) pmol/L	38.7 (CI 35.5 to 42.7)	
Neg. reference change value (RCV_{neg}) pmol/L	-27.9 (CI -29.9 to – 26.2)	
Index of individuality (II) pmol/L	0.60 (CI 0.42 to 0.78	

9. REFERENCES

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8) Røsjø et al, GISSI-HF study. Circulating secretoneurin concentrations provide independent prognostic information to established risk indices in patients with chronic heart failure. Eur Heart J, Volume 43, Issue Supplement_2, October 2022

9) SMASH 1 (Scandinavian Multicenter Study to Advance Risk Stratification in Heart Disease- Ventricular Arrhythmias: A Multicenter, Observational Trial), clinicaltrials.gov, *NCT02864771*. In-house, not published data

10) Aakre et.al, Biological variation of secretoneurin; a novel cardiovascular biomarker implicated in arrhythmogenesis. Clinical Biochemistry 2021;98:74-77

10. SYMBOLS

CE	Conformity to the European directive 98/79/EC on *in vitro* diagnostic medical devices
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
LOT	Lot number
	Expiry date (year-month-day)
	Storage temperature 2-8°C
	Manufacturer
	Consult instructions for use
Σ	Contains sufficient for <n> tests
	Do not reuse
	Warning

Example Version