Human Copeptin ELISA Kit

Enzyme Immunoassay for the quantification of human Copeptin in human serum and plasma samples

Catalog number: ARG81384

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INTRODUCTION

Copeptin (also known as CT-proAVP) is a 39-amino acid-long, glycosylated peptide derived from a pre-pro-hormone consisting of vasopressin, neurophysin II and copeptin. Arginine vasopressin (AVP), also known as the antidiuretic hormone (ADH), is involved in multiple cardiovascular and renal pathways and functions. Copeptin is synthesized mainly in the paraventricular neurons of the hypothalamus and in the supraoptical nucleus. During axonal transport, pre-pro-AVP is proteolytically cleaved into vasopressin, neurophysin II and copeptin. These molecules are then stored in secretory granules in the posterior pituitary and released upon osmotic or non-osmotic (hemodynamical; stress-related) stimuli.

The concentration of copeptin in the blood circulation ranges from 1 to 12 pmol/L in healthy individuals. The levels of copeptine are slightly higher in men than in women and are not influenced by age. In response to serum osmolality fluctuations, the kinetics of copeptine are comparable to those of vasopressin. For example, patients with an electrolyte disorders such as diabetes insipidus with very low levels of vasopressin also show very low levels of copeptin in blood plasma. On the other hand, patients suffering from syndrome of inappropriate antidiuretic hormone secretion show both high levels of vasopressin and copeptin.

Several studies have shown that copeptin is released very early during the onset of an acute myocardial infarction (AMI), raising the question of its potential value in the diagnosis of AMI and particularly in ruling-out AMI. Indeed, copeptin is released much earlier than Troponin making the interpretation of their complementary kinetics a useful tool to rule-out AMI. It has been shown that the combination of a negative result of troponin together with a negative result of copeptin can rule-out AMI at emergency department

presentation with a negative predictive value ranging from 95% to 100%. These results have been confirmed in a randomised controlled trial.
The prognostic value of vasopressin for prediction of outcome in patients suffering from heart failure has been known since the nineties. Patients presenting with high levels of vasopressin have a worsened outcome. Recently, a similar interest has been demonstrated for copeptin in heart failure.
[Wikipedia]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Copeptin in human serum and plasma samples. This assay employs the competitive quantitative enzyme immunoassay technique. A secondary antibody has been pre-coated onto a microtiter plate. The secondary antibody can bind to the Fc fragment of the primary antibody. The primary antibodies in the kit will be competitively bound by both biotinylated peptides and peptide standards or targeted peptides in samples. The wells are washed and then incubated with Streptavidin-HRP reagent. The biotinylated peptide interacts with streptavidin-horseradishperoxidase to form a complex. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Copeptin present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm±2 nm. The concentration of Copeptin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Secondary antibody coated microplate	12 x 8 wells	4°C
20X Wash Buffer	50 ml	4°C
Primary antibody	1 vial	4°C
Biotinylated peptide	1 vial	4°C
Standard	1 vial	4°C
Streptavidin-HRP complex	30 µl	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
2N HCl	15 ml (Ready-to-use)	4°C
Positive Controls (acceptable range 0.4-0.8 ng/ml)	2 vial	4°C
Plate sealer	3 pieces	Room Temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (300-400rpm)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lypholized material is at the bottom of the tube.
- If crystals are observed in the 20X Wash buffer, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 4°C at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Note: We recommended add Aprotinin (enzyme inhibitor) for **ALL** sample collection to prevent sample degradation. 0.6 TIU or 100 µl of Aprotinin per mL of sample solution.

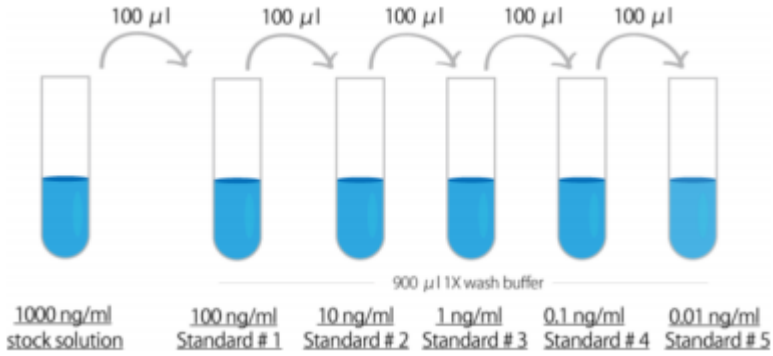
REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 20X Wash Buffer into distilled water to yield 1X Wash buffer. Keep 1X Wash Buffer at 4°C. If crystals appear in 20X Wash Buffer, warm the buffer in warm water bath (not higher than 50°C) for 30 minutes or until crystals disappear. Mix well before use.
- **Primary antibody**: Reconstitute the Primary antibody vial with 5 ml of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C.
- **Biotinylated peptide**: Reconstitute the Biotinylated peptide vial with 5 ml of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C.
- **Positive control**: Reconstitute the Positive control vial with 200 µl of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C. (acceptable range 0.4-0.8 ng/ml)
- **Streptavidin-HRP complex:** Centrifuge Streptavidin-HRP complex briefly and add 12µl of Streptavidin-HRP to 12ml 1X wash buffer to make Streptavidin-HRP solution. Vortex thoroughly.
- **Standard peptide**: Centrifuge and dilute the standard with 1 ml of 1X Wash buffer and vortex. The concentration of this stock solution is 1000 ng/ml. Allow the solution to sit for at least 10 minutes at room

temperature to completely dissolve. Dilute peptide standard solutions with 1X Wash buffer to 100 ng/ml, 10 ng/ml, 1 ng/ml, 0.1 ng/ml, 0.01 ng/ml as follows:

The example of the dilution of standards

Standard No.	Standard Conc. (ng/ml)	1X Wash Buffer (µl)	Standard (µl)
Stock	1000	-	-
S1	100	900	100 µl of Stock
S2	10	900	100 µl of S1
S3	1	900	100 µl of S2
S4	0.1	900	100 µl of S3
S5	0.01	900	100 µl of S4
S0 (Total binding)	0	900	0



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-23°C) 30 minutes before use. Standards, samples and blank should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of 1X Wash Buffer as Total Binding (zero standard). Two empty wells should be left as blank.
3. Add 50 µl of prediluted peptide standards (add from S5 to S1), 50 µl positive controls or 50 µl samples into corresponding wells. It is advisable to assay each condition in duplicates.
4. Add 25 µl of primary antibody into each well **except the Blank wells**.
5. Add 25 µl of Biotinylated peptide into each well **except the Blank wells**. It is not recommended to use a multi-channel pipette to load the primary antibody and biotinylated peptide.
6. Seal the microtiter plate with plate sealer. Incubate for 2 hours at RT. Orbital shaking at 300-400 rpm is recommended.
7. Mix and centrifuge Streptavidin-HRP vial (3,000-5,000 rpm for 5 seconds) before use. Pipette 12 µl of Streptavidin-HRP into 12 ml of 1X Wash Buffer. Vortex thoroughly. Prepare freshly.
8. Remove sealer from plate.
9. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

10. Add 100 µl of diluted Streptavidin-HRP solution into **each well**.
11. Reseal the plate with sealer. Incubate for 1 hour at RT. Orbital shaking at 300-400 rpm is recommended.
12. Remove sealer from plate. Wash as according to step 9.
13. Add 100 µl TMB substrate solution into each well.
14. Reseal the plate with sealer. Incubate for 1 hour at RT. Orbital shaking at 300-400 rpm is recommended. (Protect from light)
15. Remove sealer from plate. (**Do NOT** wash or discard the contents of the wells)

1. Add 100 µl 2N HCl into all wells to stop the reaction. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
2. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells be read within 20 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

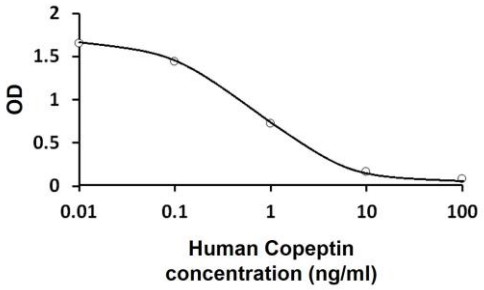
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard of Copeptin ranged from 0-100 ng/ml.

The mean MDD was 0.12 ng/ml.

Linear Range

0.12 – 2.1 ng/ml

Precision:

Intra-assay: < 10%

Inter-assay: < 15%

Cross Reactivity

The cross reactivity ratio of the tested peptide as the table:

Peptide	Cross Reactivity (%)
Copeptin (Human)	100
Copeptin (Rat)	0
[Arg^{8}]-Vasopressin (Human, Rat, Mouse, Canine)	0
Angiotensin I (Human, Rat, Mouse, Canine)	0
Angiotensin II (Human, Rat, Mouse, Porcine Canine, Rabbit, Guinea pig)	0
Endothelin-1 (Human, Rat, Mouse, Porcine, Bovine, Rabbit, Canine, Monkey)	0
Urotensin II (Human)	0

Amino acid of Copeptin:

Ala-Ser-Asp-Arg-Ser-Asn-Ala-Thr-Gln-Leu-Asp-Gly-Pro-Ala-Gly-Ala-Leu-Leu-Leu-Arg-Leu-Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-Pro-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr