Cholecystokinin Octapeptide CCK (26-33) Extraction-free ELISA Kit (For Human, Rat, Mouse)

Extraction-free Enzyme Immunoassay for the quantification of human, rat or mouse Cholecystokinin Octapeptide (CCK)(26-33) in plasma samples

Catalog number: ARG80972

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Cholecystokinin is a brain/gut peptide. In the gut, it induces the release of pancreatic enzymes and the contraction of the gallbladder. In the brain, its physiologic role is unclear. The cholecystokinin pro-hormone is processed by endo- and exo-proteolytic cleavages. Two transcript variants encoding the same protein have been found for this gene. [provided by RefSeq, Mar 2010]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique for the detection of a specific peptide. The microtiter plate is coated with secondary antibody and the non-specific binding sites are blocked. The secondary antibody can be bound by an Fc region of a primary antibody whose Fab region is competitively bound by biotinylated peptide and peptide standard or targeted peptide in samples. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of CCK (26-33) peptide in test sample is indirectly proportional to the color intensity, which can be determined by extrapolation 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	1 plate (96 well)	4°C
Primary antibody	1 vial	4°C
Standard peptide	1 vial	4°C
Biotinylated peptide	1 vial	4°C
SA-HRP concentrate	30 µl	4°C
20X Assay buffer	50 ml	4°C
Positive control	2 vials	4°C
Diluent buffer	23 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution (2N HCl)	13 ml	4°C
Plate sealer	3	RT

MATERIALS REQUIRED BUT NOT PROVIDED

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

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.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) 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

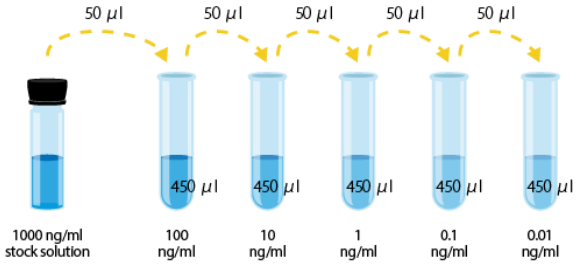
The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Assay buffer: Dilute 20X assay buffer into distilled water to yield 1X assay buffer. Mix well before use. Keep 1X assay buffer at 4°C.
- Primary antibody: Briefly spin down primary antibody before opening. Add 1ml of 1X assay buffer into primary antibody. Allow to sit for 5-10 minutes to make sure that it is completely dissolved. Next, add 4ml of diluent buffer to the vial. Allow to sit for 5 minutes. Mix well before use.
- Biotinylated peptide: Briefly spin down biotinylated peptide before opening. Add 1ml of 1X assay buffer into biotinylated peptide. Allow to sit for 5-10 minutes to make sure that it is completely dissolved. Next, add 4ml of diluent buffer to the vial. Allow to sit for 5 minutes. Mix well before use.
- Positive control: Briefly spin down vial before opening. Add 200 µl of diluent buffer into control. Allow to sit for 5-10 minutes to make sure that it is completely dissolved. Mix well before use.
- Streptavidin Horseradish Peroxidase (SA-HRP): Briefly spin down vial before opening. Add 12 µl of SA-HRP into 12ml of 1X assay buffer to make SA-HRP working solution. Mix well before use.
- Sample preparation: Dilute plasma sample 1:1 with diluent buffer. Mix well before use. It is highly recommended that normal plasma samples be used in comparison with patient plasma samples to establish a baseline value.
- Standard peptide: Briefly spin down vial before opening. Dilute standard peptide with 1ml of 1X assay buffer. Vortex. The concentration of this

stock solution is 1000 ng/ml. Allow to sit for 5-10 minutes to make sure that it is completely dissolved. Prepare peptide standard serial dilutions with diluent buffer to make standards of 100ng/ml, 10ng/ml, 1ng/ml, 0.1ng/ml, 0.01ng/ml as according to the figure below:



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Leave 2 wells as Blank.
3. Add 50 µl of diluent buffer into 2 wells as Total Binding.
4. Add 50 µl of each Standard, Control and samples into appropriate wells.
5. Add 25 µl of primary antibody into each well except the Blank wells.
6. Add 25 µl of biotinylated peptide into each well except the Blank wells.(Multichannel pipette is NOT recommended to load primary antibody and biotinylated peptide.

7. Seal and incubate plate for 2 hours at room temperature. Orbital shaking at 300-400 rpm is recommended.
8. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× assay 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 buffer by aspirating, decanting or blotting against clean paper towels.
9. Add 100 µl of SA-HRP into each well.
10. Seal and incubate plate for 1 hour at room temperature. Orbital shaking at 300-400 rpm is recommended.
11. Wash as according to step 8.
12. Add 100 µl of TMB Reagent to each well. Seal and incubate for 1 hour at room temperature in dark.
13. Add 100 µl of Stop Solution to each well.
14. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

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

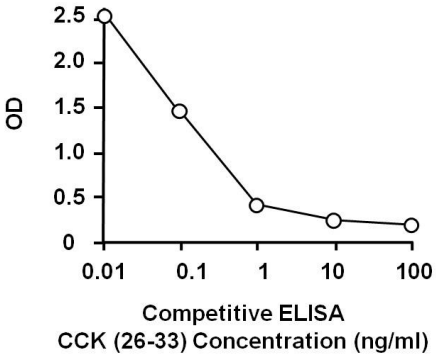
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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

Assay range: 0-100 ng/ml

Minimum Detectable Concentration: 0.04 ng/ml

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Specificity

The following substances were tested for cross reactivity of the assay:

Peptide	Cross reactivity %
CCK (26-33) (non-sulfated)	100
Big Gastrin-1	100
Caerulein	100
CCK-33	100
CCK (27-33)	100
Gastrin-1	100
CCK-33 (sulfated)	42.9
CCK (30-33)	12.8
CCK-33 (non-sulfated)	8
CCK (26-33) (sulfated)	3.9
Pancreatic Polypeptide	0
VIP	0