Datasheet for ABIN1979275

Cholecystokinin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Cholecystokinin (CCK)
Reactivity:	Human, Rat, Mouse
Method Type:	Competition ELISA
Detection Range:	0.1-1.000 pg/mL
Minimum Detection Limit:	0.1 pg/mL
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat Cholecystokinin EIA Kit optimized for serum and cell culture medium. Competition-based ELISA on a 96-well strip plate.
Sample Type:	Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Cross Reactivity: This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, NPY and APC.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, NPY and APC.
Sensitivity:	0.2 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Standard Peptide • Assay Diluent(s) • Biotinylated Peptide • HRP-Streptavidin • TMB One-Step Substrate • Stop Solution • Assay Diagram • Positive Control Sample • Capture Antibody • User Manual
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 mL volumes • Adjustable 1-25 mL pipettes for reagent preparation • 100 mL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Orbital shaker • Aluminum foil • Saran Wrap • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • SigmaPlot software (or other software that can perform four-parameter logistic regression models)

Target Details

Target:	Cholecystokinin (CCK)
Alternative Name:	CCK (CCK Products)
Background:	Cholecystokinin (CCK) is a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein. It is synthesized by I-cells in the mucosal epithelium of the small intestine and secreted in the duodenum, and causes the release of digestive enzymes from the pancreas and bile from the gallbladder. CCK is a family of hormones identified by number of amino acids depending on post-translational modification of preprocholecystokinin, including CCK58, CCK33 and CCK8. CCK is very similar in structure to another peptide hormone gastrin. They share five identical amino acids at their C-termini. CCK

Target Details

	mediates a number of physiological processes, including digestion and satiety. Secretion of CCK by the duodenal and intestinal mucosa is stimulated by fat- or protein-rich chyme entering the duodenum. It then inhibits gastric emptying and gastric acid secretion and mediates digestion in the duodenum. It stimulates the acinar cells of the pancreas to release water and ions and stimulates the secretion of a juice rich in pancreatic digestive enzymes, hence the old name pancreozymin. Together these enzymes catalyze the digestion of fat, protein, and carbohydrates. Thus, as the levels of the substances that stimulated the release of CCK drop, the concentration of the hormone drops as well. The release of CCK is also inhibited by somatostatin. CCK also causes the increased production of hepatic bile, and stimulates the contraction of the gall bladder and the relaxation of the Sphincter of Oddi (Glisson's sphincter), resulting in the delivery of bile into the duodenal part of the small intestine. Bile salts form amphipathic micelles that emulsify fats, aiding in their digestion and absorption.
Gene ID:	885
UniProt:	P06307
Pathways:	TCR Signaling, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Positive Regulation of Immune Effector Process, Positive Regulation of Endopeptidase Activity, Toll-Like Receptors Cascades, Feeding Behaviour

Application Details

Application Notes:	Recommended Dilution for serum and plasma samplesHuman: 4X / Mouse: 4X / Rat: 2X
Sample Volume:	100 µL
Assay Time:	5 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed. 2. Add 100 µL detection antibody to each well. 3. Incubate 1.5 h at RT or O/N at 4 °C. 4. Add 100 µL standard or sample to each well. 5. Incubate 2.5 h at RT. 6. Add 100 µL prepared streptavidin solution. 7. Incubate 45 min at RT. 8. Add 100 µL TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL Stop Solution to each well. 11. Read plate at 450 nm immediately.

Application Details

Reagent Preparation:	1. Keep kit reagents on ice during steps. Equilibrate plate to room temperature before opening the sealed pouch. 2. Briefly centrifuge the CCK Antibody vial (Item N) and reconstitute with 5 µL of ddH2O before use. Add 50 µL of 1x Assay Diluent E into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently. 3. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent E. This is your anti-CCK antibody working solution, which will be used in step 2 of the Assay Procedure. NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure). 4. Briefly centrifuge the vial of biotinylated CCK peptide (Item F) and reconstitute with 20 µL of ddH2O before use. Add 10 µL of Item F to 5 mL 1X Assay Diluent E. Pipette up and down to mix gently. The final concentration of biotinylated CCK will be 20 pg/mL. This solution will only be used as the diluent in step 5 of Reagent Preparation. 5. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL and 0 pg/mL. Pipette 450 mL of biotinylated CCK solution into each tube, except for the 1000 pg/mL (leave this one empty). It is very important to make sure the concentration of biotinylated CCK is 20 pg/mL in all standards. a. Briefly centrifuge the vial of standard CCK peptide (Item C) and reconstitute with 10 µL of ddH2O. In the tube labeled 1000 pg/mL, pipette 8 µL of Item C and 792 µL of 20 pg/mL biotinylated CCK solution (prepared in step 4 above). This is your CCK stock solution (1000 pg/mL CCK, 20 pg/mL biotinylated CCK). Mix thoroughly. This solution serves as the first standard. b. To make the 100 pg/mL standard, pipette 50 µL of CCK stock solution into the tube labeled 100 pg/mL. Mix thoroughly. c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 mL of biotinylated CCK and 50 mL of the prior concentration until 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer. d. The final tube (0 pg/mL CCK, 20 pg/mL biotinylated CCK) serves as the zero standard (or total binding). 6. Prepare a 10-fold dilution of Item F. To do this, add 2 mL of Item F to 18 mL of the 1X Assay Diluent E. This solution will be used in steps 7 and 9. 7. Positive Control Preparation: Briefly centrifuge the positive control vial and reconstitute with 100 µL of ddH2O before use (Item M). To the tube of Item M, add 101 µL 1x Assay Diluent E. Also add 4 µL of 10-fold diluted Item F (prepared in step 6) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample that is meant to be a system control (to verify that the detection & kit components are working). It may be diluted further if desired, but be sure the final concentration of biotinylated CCK is 20

	pg/mL. 8. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer. 1000 100 10 1 0.1 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 50 mL 50 mL 50 mL 50 mL 9. Sample Preparation: Use 1X Assay Diluent E + biotinylated CCK to dilute samples, including serum/plasma, cell culture medium and other sample types. It is very important to make sure the final concentration of the biotinylated CCK is 20 pg/mL in every sample. Example: to make a 4-fold dilution of sample, mix together 5 µL of 10-fold diluted Item F (prepared in step 6), 182.5 mL of 1X Assay Diluent E, and 62.5 µL of your sample, mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated CCK to a final concentration of 20 pg/mL. Example: Add 5 mL of 10-fold diluted Item F to 245 mL of sample. 10. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 80- fold with 1X Assay Diluent E.
Sample Preparation:	Use 1X Assay Diluent E + biotinylated CCK to dilute samples, including serum/plasma, cell culture medium and other sample types. It is very important to make sure the final concentration of the biotinylated CCK is 20 pg/mL in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 5 µL of 10-fold diluted Item F (prepared in step 6), 182.5 mL of 1X Assay Diluent E, and 62.5 µL of your sample, mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated CCK to a final concentration of 20 pg/mL. EXAMPLE: Add 5 mL of 10-fold diluted Item F to 245 mL of sample.
Assay Procedure:	1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL anti-CCK antibody (see Reagent Preparation step 3) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4 degrees C. 0 3. Discard the solution and wash wells 4 times with 1X Wash Buffer (200-300 µL each) Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of each standard (see Reagent Preparation step 5), positive control (see Reagent

Application Details

	Preparation step 7) and sample (see Reagent Preparation step 9) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4 °C. 5. Discard the solution and wash 4 times as directed in Step 3. 6. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation step 10) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes. 7. Discard the solution and wash 4 times as directed in Step 3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec). 9. Add 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance on the y-axis. Draw the best-fit curve through the standard points.
Assay Precision:	Intra-Assay: CV < 10 % Inter-Assay: CV < 15 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze/thaw cycles.
Storage:	-20 °C
Storage Comment:	Standard, Biotinylated CCK peptide, and Positive Control should be stored at -20°C after arrival. Avoid multiple freeze-thaws. The remaining kit components may be stored at 4°C. Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
Expiry Date:	6 months

Publications

Product cited in:	Genton, Pruijm, Teta, Bassi, Cani, Gaïa, Herrmann, Marangon, Mareschal, Muccioli, Stoermann,

Suriano, Wurzner-Ghajarzadeh, Lazarevic, Schrenzel: "Gut barrier and microbiota changes with glycine and branched-chain amino acid supplementation in chronic haemodialysis patients." in: **Journal of cachexia, sarcopenia and muscle**, (2021) (PubMed).

Zhou, Yang, Lin, Okoro, Guo: "Cholecystokinin elevates mouse plasma lipids." in: **PLoS ONE**, Vol. 7, Issue 12, pp. e51011, (2013) (PubMed).

Reis, Ribeiro, Costa, Bressan, Alfenas, Mattes: "Acute and second-meal effects of peanuts on glycaemic response and appetite in obese women with high type 2 diabetes risk: a randomised cross-over clinical trial." in: **The British journal of nutrition**, Vol. 109, Issue 11, pp. 2015-23, (2013) (PubMed).

Miyamoto, Shikata, Miyasaka, Okada, Sasaki, Kodera, Hirota, Kajitani, Takatsuka, Kataoka, Nishishita, Sato, Funakoshi, Nishimori, Uchida, Ogawa, Makino: "Cholecystokinin plays a novel protective role in diabetic kidney through anti-inflammatory actions on macrophage: anti-inflammatory effect of cholecystokinin." in: **Diabetes**, Vol. 61, Issue 4, pp. 897-907, (2012) (PubMed).

Gurda, Guo, Lee, Molkentin, Williams: "Cholecystokinin activates pancreatic calcineurin-NFAT signaling in vitro and in vivo." in: **Molecular biology of the cell**, Vol. 19, Issue 1, pp. 198-206, (2008) (PubMed).

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