

Product Information

Cholecystokinin (CCK) EIA Kit

for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0039**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

The Cholecystokinin (CCK) Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting CCK peptide based on the principle of competitive enzyme immunoassay. In this assay, a biotinylated CCK peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated CCK peptide competes with endogenous (unlabelled) CCK for binding to the anti-CCK antibody. After a wash step, any bound biotinylated CCK then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated CCK peptide and inversely proportional to the amount of endogenous CCK in the standard or samples. A standard curve of known concentration of CCK peptide can be established and the concentration of CCK peptide in the samples can be calculated accordingly.

Components

1. 96 well plate coated with secondary antibody (Item A) - RAB0039A: 96 wells (12 strips x 8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL
3. EIA Cholecystokinin Peptide standard, Lyophilized (Item C) - RAB0039C: 2 vials
4. Anti-CCK Detection Antibody, Lyophilized (Item N) - RAB0039F: 2 vials
5. EIA CCK 5x Assay Diluent B (Item E) – RABDIL10: 15 mL of 5x concentrated buffer. Diluent for both standards and samples, including serum or plasma, cell culture media, or other sample types.
6. Biotinylated CCK Peptide, Lyophilized (Item F) - RAB0039G: 2 vials
7. HRP-streptavidin (Item G) - RABHRP3: 600 μL of 40x concentrated HRP-conjugated Streptavidin.
8. CCK Positive Control Sample, Lyophilized (Item M) - RAB0039K: 1 vial

9. TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
10. Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 μL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Ultrapure water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminium foil
11. Plastic wrap

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

For sample and positive control dilutions, refer to Preparation, steps 6, 7, and 8.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. 5x Assay Diluent B (Item E) should be diluted 5-fold with ultrapure water.
3. Briefly centrifuge the anti-CCK Antibody vial (Item N) and reconstitute with 55 μL of 1x Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B (Item E). This is the anti-CCK antibody working solution, which will be used in Assay Procedure, step 2.

Note: The following steps may be done during the antibody incubation procedure (Procedure, step 2).

5. Briefly centrifuge the vial of biotinylated CCK peptide (Item F) and reconstitute with 2 μL of ultrapure water before use. Transfer the entire contents of the Item F vial into a tube containing 5 mL of 1x Assay Diluent B. This is the Working Stock of Item F. Pipette up and down to mix gently. The final concentration of biotinylated CCK will be 40 pg/mL. This solution will be used as the diluent in Preparation, steps 6, 7, and 9.

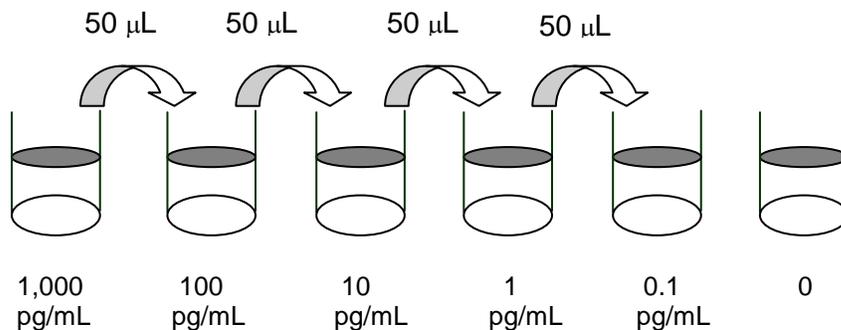
6. **Preparation of Standards:** Label 6 microtubes with the following concentrations: 1,000 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL, and 0 pg/mL. Pipette 450 μL of biotinylated CCK Item F working solution (prepared in Step 5) into each tube, except for the 1,000 pg/mL (leave this one empty).

Prepare a 2-fold dilution of Item F. To do this, add 100 μL of Working Stock Item F (see Preparation, step 5) to 100 μL of the prepared Positive Control (Item M). The final concentration of biotinylated CCK will be **20 pg/mL**.

Note: It is very important to make sure the concentration of biotinylated CCK is 20 pg/mL in all standards.

- Briefly centrifuge the vial of standard CCK peptide (Item C) and reconstitute with 10 μL of ultrapure water. In the tube labeled 1,000 pg/mL, pipette 8 μL of Item C and 792 μL of 20 pg/mL biotinylated CCK solution prepared above. This is the CCK stock solution (1,000 pg/mL CCK and 20 pg/mL biotinylated CCK). Mix thoroughly. This solution serves as the first standard.
- To make the 100 pg/mL standard, pipette 50 μL of 1,000 pg/mL CCK stock solution into the tube labeled 100 pg/mL. Mix thoroughly.
- Repeat this step with each successive concentration, preparing a dilution series as shown in Figure 1. Each time, use 450 μL of biotinylated CCK and 50 μL of the prior concentration until 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer.
- The final tube (0 pg/mL CCK and 20 pg/mL biotinylated CCK) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



7. **Positive Control Preparation:** Briefly centrifuge the positive control vial (Item M) and reconstitute with 100 μL of ultrapure water before use. Add 100 μL of Working Stock Item F (see Preparation, step 5) to 100 μL of the prepared Positive Control (Item M). Mix thoroughly. This is a 2-fold dilution of the Positive Control. The positive control is a cell culture medium sample with an expected signal between 10–30% of total binding (70–90% competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated CCK is 20 pg/mL .

8. **Sample Preparation:** Use 1x Assay Diluent E plus biotinylated CCK to dilute samples, including serum/plasma, cell culture medium, and other sample types.

Note: It is very important to make sure the final concentration of the biotinylated CCK is 20 pg/mL in every sample. To perform a 2-fold dilution of the sample, add 125 μL of Working Stock Item F (see Preparation, step 5) to 125 μL of prepared sample. The final concentration of biotinylated CCK will be **20 pg/mL** . For a higher dilution of the sample, dilute the sample with the appropriate Assay Diluent before performing above step.

For example: to make a 4-fold dilution of sample, dilute sample 2-fold (62.5 μL of sample plus 62.5 μL of 1x Assay Diluent B). Mix together 125 μL of 2-fold diluted Item F (Preparation, step 6), 125 μL of the prepared sample; mix gently. The total volume is 250 μL , enough for duplicate wells on the microplate.

Do not use Item F diluent from Preparation, step 6 for sample preparation.

9. 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 ultrapure water to yield 400 mL of 1x Wash Buffer.
10. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 40-fold with 1x Assay Diluent B.

Storage/Stability

The Standard, Biotinylated CCK peptide, and Positive Control should be stored at $-20\text{ }^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

The remaining kit components may be stored at $2\text{--}8\text{ }^{\circ}\text{C}$.

Opened microplate strips and antibody (Item N) may be store for up to 1 month at $2\text{--}8\text{ }^{\circ}\text{C}$. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

The kit remains active for up to 6 months.

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 of anti-CCK antibody (Item N) (see Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at $4\text{ }^{\circ}\text{C}$.
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 Preparation, step 6), positive control (see Preparation, step 7) and sample (see Preparation, step 8) 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) overnight or at $4\text{ }^{\circ}\text{C}$.
5. Discard the solution and wash 4 times as directed in step 3.
6. Add 100 μL of prepared HRP-Streptavidin solution (see 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 μ L 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 μ L of Stop Solution (Item I) to each well. Read absorbance at 450 nm immediately.

Results

Calculations

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 (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

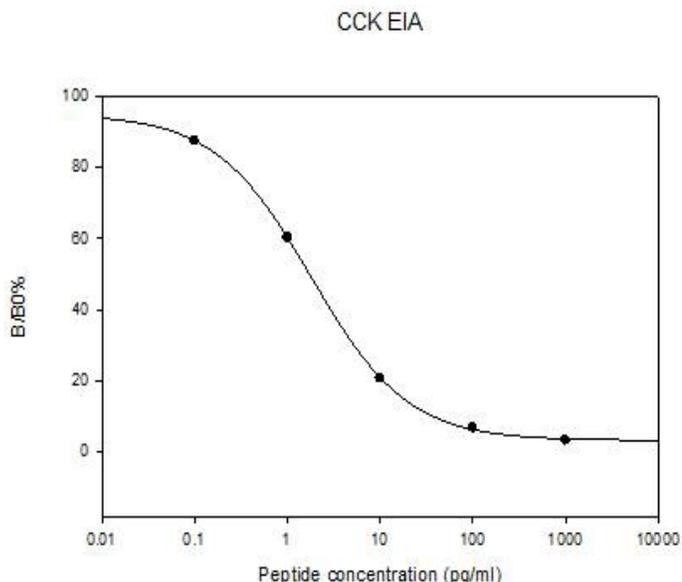
$$\text{Percentage absorbance} = \frac{(B - \text{blank OD})}{(B_0 - \text{blank OD})}$$

B = OD of sample or standard

B₀ = OD of zero standard (total binding)

Typical Data

Standard curve is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of CCK is 0.2 pg/mL.

Reproducibility:

Intra-Assay: CV <10%

Inter-Assay: CV <15%

Detection Range: 0.1–1,000 pg/mL

Specificity

Cross-Reactivity: This kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, NPY, and APC.

References

1. Greenough, A. et al., Untangling the effects of hunger, anxiety, and nausea on energy intake during intravenous cholecystokinin octapeptide (CCK-8) infusion. *Physiol. Behav.*, **65**(2), 303–310 (1998).
2. Fink, H. et al., Major biological actions of CCK--a critical evaluation of research findings. *Exp. Brain Res.*, **123**(1-2), 77–83 (1998).
3. Galés, C. et al., Identification of tyrosine 189 and asparagine 358 of the cholecystokinin 2 receptor in direct interaction with the crucial C-terminal amide of cholecystokinin by molecular modeling, site-directed mutagenesis, and structure/affinity studies. *Mol. Pharmacol. (United States)*, **63**(5), 973–982 (2003).
4. Gurda, G.T. et al., Cholecystokinin Activates Pancreatic Calcineurin-NFAT Signaling *In Vitro* and *In Vivo*. *Mol. Biol. Cell (United States)*, **19**(1), 198–206 (2008).

Appendix
Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at ≤ -20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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