**Cholecystokinin (CCK) EIA Kit**

for serum, plasma, culture supernatant, and cell lysates

**Catalog Number** RAB0039

**Storage Temperature** –20 °C

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**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, 8, and 9.

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 20 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 and 8.

6. Prepare a 2-fold dilution of Item F. To do this, add 100 µL of Working Stock Item F to 100 µL of the prepared Positive Control (Item M). The final concentration of biotinylated CCK will be 20 pg/mL. This solution will be used in Preparation, steps 8 and 10.

7. **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).

**Note:** 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 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 (Preparation, step 6). 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.

b. 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.

c. 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.

d. 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
8. **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 to 100 μL of the prepared Positive Control (Item M). Mix thoroughly. 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.

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. **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. 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 7 for sample preparation.

11. 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 °C. Avoid repeated freeze-thaw cycles.

The remaining kit components may be stored at 2–8 °C.

Opened microplate strips and antibody (Item N) may be store for up to 1 month at 2–8 °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 °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 7), positive control (see Preparation, step 8) and sample (see Preparation, step 10) 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 °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 11) 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 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.

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


## Appendix

### Troubleshooting Guide

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