

## Product Information

### Apelin EIA Kit

for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0018**

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

## TECHNICAL BULLETIN

### Product Description

The Apelin C-Terminus Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting Apelin C-Terminus peptide based on the principle of competitive enzyme immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Apelin C-Terminus antibody, both biotinylated Apelin C-Terminus peptide, and peptide standard or targeted peptide in samples interacts competitively with the Apelin C-Terminus antibody. Uncompeted (bound) biotinylated Apelin C-Terminus peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of Apelin C-Terminus peptide in the standard or samples. This is due to the competitive binding to Apelin C-Terminus antibody between biotinylated Apelin C-Terminus peptide and peptides in standard or samples. A standard curve of known concentration of Apelin C-Terminus peptide can be established and the concentration of Apelin C-Terminus peptide in the samples can be calculated accordingly.

### Components

1. 96-well plate coated with secondary antibody (Item A) - RAB0018A: 96 wells (12 strips  $\times$  8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL.
3. Apelin peptide standard, Lyophilized (Item C) - RAB0018C: 2 vials.
4. Anti-Apelin Detection Antibody, Lyophilized (Item N) - RAB0018F: 2 vials.
5. 5x Assay Diluent B (Item E) - RABDIL10: 2 vials, 25 mL/vial. Diluent for both standards and samples including serum or plasma, cell culture media, or other sample types.
6. Biotinylated Apelin Peptide, Lyophilized (Item F) - RAB0207G: 2 vials.

7. HRP-Streptavidin concentrate (Item G) - RABHRP3: 600  $\mu\text{L}$  of 100x concentrated HRP-conjugated Streptavidin.
8. Apelin Positive Control Sample, Lyophilized (Item M) - RAB0207K: 1 vial.
9. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3',5,5'- tetramethylbenzidine (TMB) in buffered solution.
10. Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.

### Reagents and Equipment Required but Not Provided.

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2  $\mu\text{L}$  to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- SigmaPlot software (or other software which can perform four-parameter logistic regression models).
- Tubes to prepare standard or sample dilutions.
- Orbital shaker.
- Aluminum foil.

### 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 steps 6, 7, 8, and 10 of Preparation.

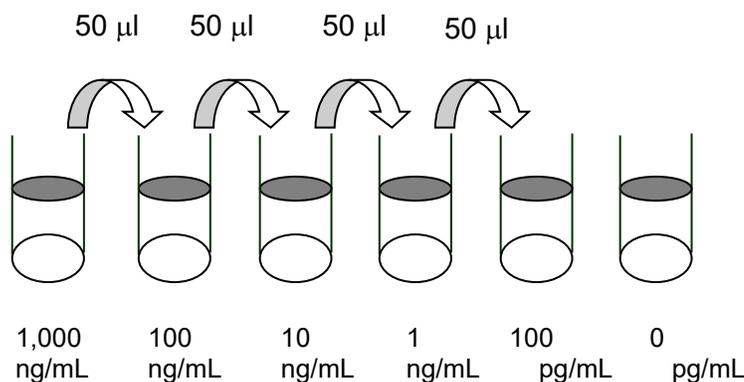
1. Keep kit reagents on ice during preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. 5x Assay Diluent B (Item E) should be diluted 5-fold with distilled water.
3. Briefly centrifuge the Anti-Apelin C-Terminus Antibody vial (Item N) and reconstitute with 5  $\mu\text{L}$  of water before use. Add 50  $\mu\text{L}$  of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.
4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is the anti-Apelin C-Terminus antibody working solution, which will be used in 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 Apelin C-Terminus (Item F) and reconstitute with 20  $\mu\text{L}$  of water before use. Add 5  $\mu\text{L}$  of Item F to 5 mL of the 1x Assay Diluent B. Pipette up and down to mix gently. The final concentration of biotinylated Apelin C-Terminus will be 50 ng/mL. This solution will only be used as the diluent in Preparation, step 6.
  6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL, and 0 pg/mL. Pipette 450  $\mu\text{L}$  of biotinylated Apelin C-Terminus solution into each tube, except for the 1,000 ng/mL (leave this one empty).
- Note: It is very important to make sure the concentration of biotinylated Apelin C-Terminus is 50 ng/mL in all standards.
- a. Briefly centrifuge the vial of Apelin C-Terminus (Item C) and reconstitute with 10  $\mu\text{L}$  of water. In the tube labeled 1,000 ng/mL, pipette 8  $\mu\text{L}$  of Item C and 792  $\mu\text{L}$  of 50 ng/mL biotinylated Apelin C-Terminus solution (Preparation, step 5). This is the Apelin C-Terminus stock solution (1,000 ng/mL Apelin C-Terminus and 50 ng/mL biotinylated Apelin C-Terminus). Mix thoroughly. This solution serves as the first standard.
  - b. To make the 100 ng/mL standard, pipette 50  $\mu\text{L}$  of Apelin C-Terminus stock solution into the tube labeled 100 ng/mL. Mix thoroughly.
  - c. Repeat this step with each successive concentration, preparing a dilution series (see Figure 1). Each time, use 450  $\mu\text{L}$  of biotinylated Apelin C-Terminus and 50  $\mu\text{L}$  of the prior concentration until 100 pg/mL is reached. Mix each tube thoroughly before the next transfer.
  - d. The final tube (0 pg/mL Apelin C-Terminus, 50 ng/mL biotinylated Apelin C-Terminus) serves as the zero standard (or total binding).

**Figure 1.**  
Dilution Series for Standards



7. Prepare a 10-fold dilution of Item F. To do this, add 2  $\mu\text{L}$  of Item F to 18  $\mu\text{L}$  of the 1x Assay Diluent B. This solution will be used in Preparation, steps 8 and 10.
8. **Positive Control Preparation:** briefly centrifuge the positive control vial (Item M) and reconstitute with 100  $\mu\text{L}$  of water before use. To the tube of Item M, add 101  $\mu\text{L}$  of 1x Assay Diluent B. Also add 2  $\mu\text{L}$  of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. 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 Apelin C-Terminus is 50 ng/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 deionized or distilled water to yield 400 mL of 1x Wash Buffer.
10. **Sample Preparation:** Use 1x Assay Diluent B and biotinylated Apelin C-Terminus to dilute samples, including serum/plasma, cell culture medium, and other sample types. For example, to make a 4-fold dilution of sample, mix together 2.5  $\mu\text{L}$  of 10-fold diluted Item F (Preparation, step 7), 185  $\mu\text{L}$  of 1x Assay Diluent B, and 62.5  $\mu\text{L}$  of sample; mix gently. The total volume is 250  $\mu\text{L}$ , enough for duplicate wells on the microplate.

**Note:** It is very important to make sure the final concentration of the biotinylated Apelin C-Terminus is 50 ng/mL in every sample.

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

If undiluted samples are used, biotinylated Apelin C-Terminus must still be added to a final concentration of 50 ng/mL. For example, add 2.5  $\mu\text{L}$  of 10-fold diluted Item F to 247.5  $\mu\text{L}$  of sample.

Optimal sample dilution factors should be determined empirically.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 100-fold with 1x Assay Diluent B.

### Storage/Stability

Standard, Biotinylated Apelin C-Terminus 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  $4\text{ }^{\circ}\text{C}$ .

Opened microplate strips and Item N may be stored 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 1 year.

### 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  $\mu\text{L}$  of anti-Apelin C-Terminus antibody (see Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or overnight at  $4\text{ }^{\circ}\text{C}$ .
3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200–300  $\mu\text{L}$  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  $\mu\text{L}$  of each standard (see Preparation, step 6), 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) or overnight at  $4\text{ }^{\circ}\text{C}$ .
5. Discard the solution and wash 4 times as directed in step 3.
6. Add 100  $\mu\text{L}$  of prepared HRP-Streptavidin solution (see Preparation, step 11) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 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  $\mu$ 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  $\mu$ L of Stop Solution (Item I) to each well. Read absorbances 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.

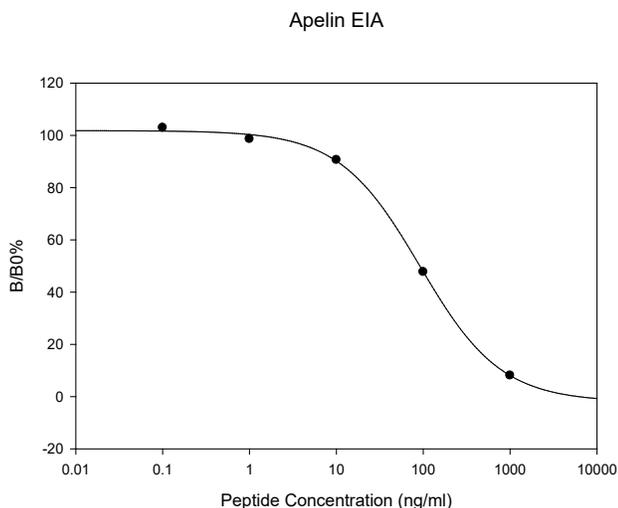
$$\text{Percentage absorbance} = \frac{(B - \text{blank OD})}{(B_0 - \text{blank OD})}$$

B = OD of sample or standard

B<sub>0</sub> = 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 Apelin C-Terminus is 5.84 pg/mL.

**Reproducibility:**

Intra-Assay: CV <10%

Inter-Assay: CV <15%

**Detection Range:**

0.1-1,000 ng/mL

## Specificity

This kit is designed to target the C-terminus of the 77 amino acid apelin peptide and therefore, is expected to detect all active Apelin peptides, including Apelin-13, Apelin-28, Apelin-31, and Apelin-36.

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

## References

1. Principe, A. et al., The hepatic apelin system: A new therapeutic target for liver disease. *Hepatology*, **48**(4), 1193-1201 (2008).
2. Lee, D.K. et al., Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol. Sci.*, **27**(4), 190–194 (2006).
3. Lee, D.K. et al., Modification of the terminal residue of apelin-13 antagonizes its hypotensive action. *Endocrinology*, **146**(1), 231–236 (2005).
4. Lee, D.K. et al., Agonist-independent nuclear localization of the Apelin, angiotensin AT1, and bradykinin B2 receptors. *J. Biol. Chem.*, **279**(9), 7901–7908 (2004).
5. Lee, D.K. et al., Characterization of apelin, the ligand for the APJ receptor. *J. Neurochem.*, **74**(1), 34–41 (2000).

**Appendix**  
Troubleshooting Guide

| <b>Problem</b>      | <b>Cause</b>                                    | <b>Solution</b>  |
|---------------------|---|--|
| 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 $\leq -20$ °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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