DRG® Apelin C-Terminus Elisa (EIA-5178)

As of 2 May 2011 rm (Vers. 1.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

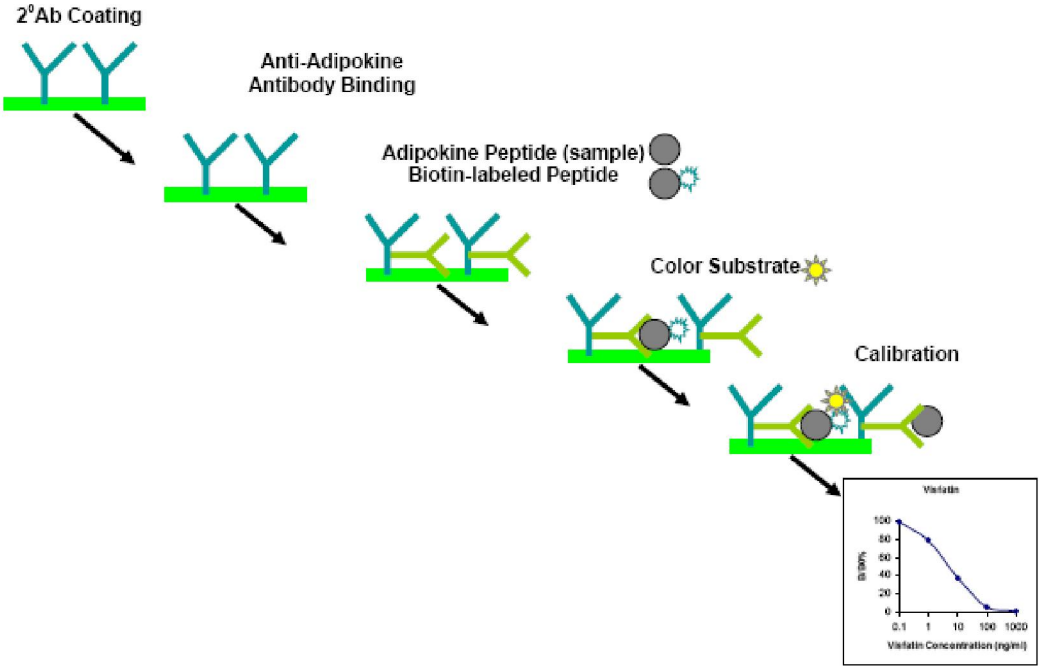
Not for use in diagnostic procedures.

1 GENERAL DESCRIPTION

The Apelin-C Terminus Enzyme Immunoassay (EIA) Kit is an 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.

How does the EIA work?



2 REAGENTS

1. **Apelin-C Terminus Microplate** (Item A):
   96 wells (12 strips x 8 wells) coated with secondary antibody.
2. **Wash Buffer Concentrate (20x)** (Item B):
   25 ml
3. **Standard Apelin-C Terminus Peptide** (Item C):
   2 vials
4. **Anti-Apelin-C Terminus polyclonal antibody** (Item N):
   2 vials
5. **Assay Diluent A** (Item D):
   30 ml, contains 0.09% sodium azide as preservative.
   For Standard/Sample (serum/plasma) diluent.
6. **Assay Diluent B** (Item E):
   15 ml of 5x concentrated buffer.
   For Standard/Sample (cell culture medium/urine) diluent.
7. **Biotinylated Apelin-C Terminus peptide,** (Item F):
   2 vials
8. **HRP-Streptavidin concentrate** (Item G):
   8 µl 5,000 X concentrated HRP-conjugated Streptavidin.
9. **Positive control** (Item M):
   1 vial
10. **TMB One-Step Substrate Reagent** (Item H):
    12 ml of 3, 3', 5, 5'¬tetramethylbenzidine (TMB) in buffered solution.
11. **Stop Solution** (Item I):
    8 ml of 2 M sulfuric acid.
12. **Assay Diagram** (Item J).
13. **User Manual** (Item K)

3 STORAGE

The kit may be stored **for up to 6 months at -20 °C from the date of shipment**.

Standard, Biotinylated Apelin-C Terminus peptide, and positive control should be stored at -20 °C or -80 °C (recommended at –80 °C) after arrival.

Opened Microplate Wells and antibody may be stored for up to 1 month at 2 °C to 8 °C.

Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Avoid multiple freeze-thaws for Standard, Biotinylated Apelin-C Terminus peptide and positive control.

4 ADDITIONAL MATERIALS REQUIRED

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. Distilled or deionized 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. Aluminum foil
11. Saran Wrap

5 REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. **Assay Diluent B** (Item E)
   should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the **Anti-Apelin-C Terminus Antibody vial** (Item N) before use.
   Add 50 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
   Pipette up and down to mix gently.
   The Anti-Apelin-C Terminus antibody concentrate should be diluted 100-fold with 1x Assay Diluent B.
   This is your anti-Apelin-C Terminus antibody working solution, which will be used in step 2 of Part 7 Assay Procedure.

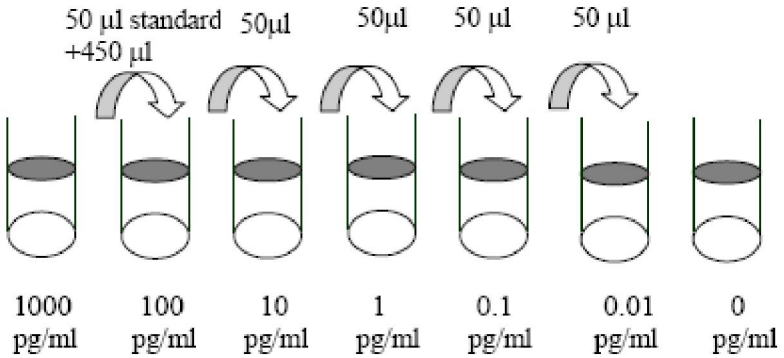
4. Briefly centrifuge the vial of **Biotinylated Apelin-C Terminus** (Item F) before use.

   Add 5 µl of biotinylated Apelin-C Terminus (Item F) to 5 ml of Assay Diluent A (if using serum/plasma samples) or

   1X Assay Diluent B (if using cell culture medium/urine samples).

   Pipette up and down to mix gently.
   The final concentration of biotinylated Apelin-C Terminus will be 50 pg/ml.
   This solution will be used in step 4 of Part 6 Reagent Preparation, which will be used as the standard diluent.
   For sample and positive control dilutions, refer to steps 5 and step 6 of Part 6 Reagent Preparation.

5. **Preparation of standard:**
Briefly centrifuge standard Apelin-C Terminus vial (Item C).
In a separate tube, pipette 10 µl of standard Apelin-C Terminus Peptide (Item C) into 990 µl of biotinylated Apelin-C Terminus solution (prepared in step 3 above) to prepare a 1000 pg/ml standard.
Pipette up and down to mix gently.
Pipette 50 µl of 1000 pg/ml Apelin-C Terminus standard into a tube with 450 µl of biotinylated Apelin-C Terminus solution.
This will be your stock standard solution (100 pg/ml Apelin-C Terminus, 50 pg/ml biotinylated Apelin-C Terminus).
Pipet 450 µl of biotinylated Apelin-C Terminus solution into 6 tubes.
Use the stock standard to produce a dilution series (shown below).
Mix each tube thoroughly before the next transfer.
Biotinylated Apelin-C Terminus serves as the zero standard (0 pg/ml), or total binding.



6. **Sample dilution**:
Use Assay Diluent A + biotinylated Apelin-C Terminus for serum/plasma samples.
For cell culture medium and urine samples, use 1X Assay Diluent B + biotinylated Apelin-C Terminus as the diluent.
It is very important to make sure the final concentration of the biotinylated Apelin-C Terminus is 50 pg/ml in all diluted samples.

For example:
For a 4-fold dilution of sample:
First make a 1:10 dilution of 50 ng/ml biotinylated Apelin-C Terminus peptide (Item F) by adding 2 µl of Item F to 18 µl of appropriate Assay Diluent; pipette up and down to mix gently.
In a separate tube, pipette 148 µl of appropriate Assay Diluent, 2 µl of 1:10 biotinylated Apelin-C Terminus, and 50 µl of your biological sample; mix gently.

7. **Positive control dilution:** 20X-40X dilution is recommended.

For example:
For a 40-fold dilution of positive control:
First make a 1:10 dilution of 50 ng/ml biotinylated Apelin-C Terminus peptide (Item F) by adding 2 µl of Item F to 18 µl of appropriate Assay Diluent; pipette up and down to mix gently.
In a separate tube, pipette 193 µl of appropriate Assay Diluent, 2 µl of 1:10 biotinylated Apelin-C Terminus, and 5 µl of your positive control (item M); mix gently.
Put the unused positive control vials back at -80°C freezer. Avoid multiple freeze-thaws.

8. If the **20X Wash Concentrate** (Item B) 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.

9. Briefly spin the **HRP-Streptavidin concentrate vial** (Item G) before use.
   HRP-Streptavidin concentrate should be diluted 5,000-fold with 1X Assay Diluent B.

   For example:
   For 10000-fold Dilution of HRP- Streptavidin solution. Briefly spin the vial (Item G) and pipette up and down to mix gently.
   Add 2 µl of HRP-Streptavidin concentrate into a tube with 198 µl 1X Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution **(don't store the diluted solution for next day use).**
   Mix thoroughly and then pipette100 µl of prepared 100-fold diluted solution into a tube with 10 ml 1x Assay Diluent B to prepare a final 10,000 fold diluted HRP-Streptavidin solution.

6 ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl anti-Apelin-C Terminus antibody (see Reagent Preparation step 3) to each well and incubate for 1.5 hours.
3. Discard the solution and wash wells 5 times with 1x Wash Solution (200 µl each).
4. Add 100 µl of each standard (see Reagent Preparation step 5), positive control (see Reagent Preparation step 7) and sample into appropriate wells.
   Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C.
5. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each).
6. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 9) to each well.
   Incubate for 45 minutes at room temperature.
7. Discard the solution and wash 5 times with 1x Wash Solution (200 µl each).
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.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

7 ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl anti-Apelin-C Terminus antibody (1000X dilution) to each well
   Incubate 1.5 hours at room temperature.
3. Add 100 µl standard peptides or sample mixed with biotinylated Apelin-C Terminus peptide to each well. Incubate 2.5 hours at room temperature or overnight at 4 °C.
4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately

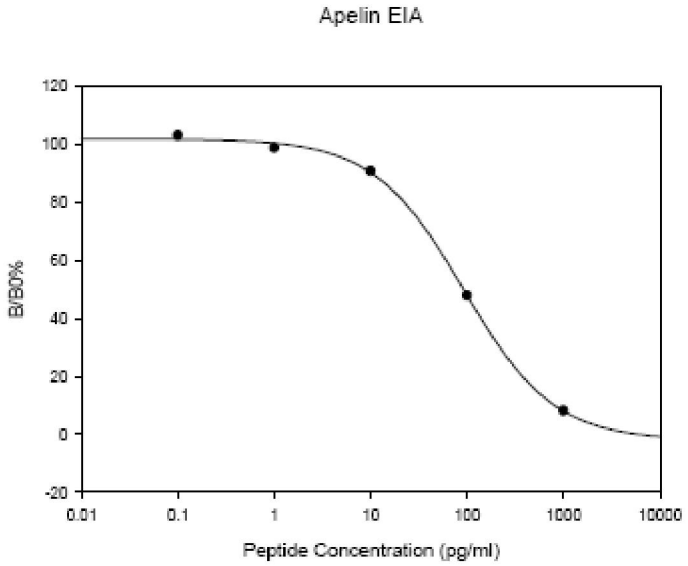
8 CALCULATION OF RESULTS

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

Percentage absorbance = (B – blank OD) / (B_0 – blank OD)
where B = OD of sample or standard and
B_0 = OD of zero standard (total binding)

8.1 TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



9 REFERENCES

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10 TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the EIA kit 2. Stop solution	1. Store your standard at<-20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

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