RETICULOCALBIN 2 (RCN2) /RAPTIN MOUSE ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RCN2/RAPTIN CONCENTRATIONS IN MOUSE SERUM, PLASMA AND CELL CULTURES

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**RCN2/RAPTIN MOUSE ELISA KIT**
Catalog No.	SK00168-12
Lot No.	
Formulation	3 x 96 T
Standard range	31.25 - 8000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Animal free Cell Cultures
Dilution Factor	***(Optimal dilutions should be determined by each laboratory for each application)***
Specificity	Mouse, Human RCN2 and Raptin
Calibration	rh RCN2 (HEK293)
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 8 months, see page 2 for more information
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
1289 HAMMERWOOD AVE SUITE-B
SUNNYVALE CA94089
USA
Tel: (408) 982 0300
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

Raptin is a sleep-inducible hormone cleaved from the RCN2 protein. Mouse Raptin is a peptide fragment derived from the Mouse RCN2 (31-252) or human Raptin is a peptide fragment of human RCN2 (28-249). The Raptin is a circulating biomarker for obesity and metabolism research. Nonsense mutations in RCN2 can lead to Night Eating Syndrome (NES) and severe obesity[1].

This High Sensitivity Mouse RCN2/Raptin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse RCN2/Raptin from serum, plasma and cell cultures in a sandwich ELISA format.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for Raptin/RCN2. The capture antibody can bind to the Raptin/RCN2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Raptin/RCN2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of Raptin/RCN2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
RCN2 Microplate - 96 well microplate coated with an antibody against RCN2.	**168-12-01**	**1 plate**
RCN2 Standard – 16 ng per vial of rh RCN2 (HEK293) in lyophilized.	**168-12-02**	**1 vial**
Detection Antibody Concentrate – 0.8 mL/vial, 10-fold concentrated of biotinylated antibody lyophilized.	**168-12-03**	**2 vials**
Positive Control- one vial of recombinant RCN2; lyophilized.	**168-12-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered based solution with preservative.	**DB98**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered based solution with preservative.	**DB90**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered peptide based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.125M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200 – 250 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Bioscience's Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Mouse Serum samples require 2- fold dilution by Dilution Buffer DB98.
Mouse EDTA plasma samples need 2~ 8 fold pre-dilution with Aviscera Bioscience's Dilution Buffer DB98.
A suggested 2-fold dilution is 50 µl sample per well + 50 µl Dilution Buffer DB98. A suggested 4-fold dilution is 25 µl per well of sample + 75 µl per well of Dilution Buffer DB98. A suggested 8-fold dilution is 12.5 µl per well of sample + 87.5 µl per well of Dilution Buffer DB98.
If the level of Raptin/RCN2 in samples is higher than 32 ng/mL, may require more fold pre-dilution.

Mouse and Bovine raptin sequence similarity or identical is over 90%, please do not use BSA or fetal bovine serum to pre-dilute the mouse samples.

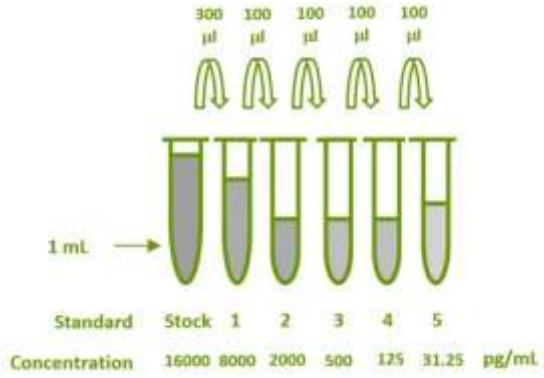
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

RCN2 Standard - Reconstitute the RCN2 standard with 1 mL of Dilution Buffer DB98. This reconstitution produces a stock solution of 16000 pg/mL (16 ng/ml). Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into the tube #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **8000 pg/mL** standard serves as the high standard. The Dilution Buffer DB98 serves as the zero standard (0 pg/mL). Store the stock solution of standard at -20 ~ - 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	16000 pg/ml
# 1	300 µl of stock	300 µl	8000 pg/mL
# 2	100 µl of 1	300 µl	2000 pg/ml
# 3	100 µl of 2	300 µl	500 pg/ml
# 4	100 µl of 3	300 µl	125 pg/ml
# 5	100 µl of 4	300 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer DB98 to prepare the working solution of positive control. Discard the working solution of positive control after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 0.8 mL of **Antibody Diluent Solution (DB90)** to produce a 10-fold concentrated stock solution.

For 96 wells test, **freshly** pipette 9.45 mL of **Antibody Diluent Solution (DB90)** into a 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. The 1 x working solution should be used in 10 min.

*If run a partial strip test, **freshly** prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.*

Streptavidin-HRP Conjugate – For 96 wells test **freshly** pipette 11.88 mL of **HRP Diluent solution (DB08C)** into a 15 mL centrifuge tube and transfer **120 µL** of **100-fold concentrated** stock solution to prepare working solution *prior to Step 7*. **Protect from light**.

*The working solution of Streptavidin-HRP Conjugate **should be freshly prepared** and used within 10-15 min. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 100-fold concentrated* Streptavidin *HRP ONLY at 2 -8°C for 12 months.*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for ***2 hours*** on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for ***90 minutes*** on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for ***45 minutes*** on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for ***10 ~18 minutes*** on microplate shaker at room temperature. **Protect from light.**
10. Add 80 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color

change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well using a microplate reader set to 450 nm within 5 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human RCN2 His Tag (HEK293)	100
Mouse RCN2 (31-252) His Tag (E. Coli)	100

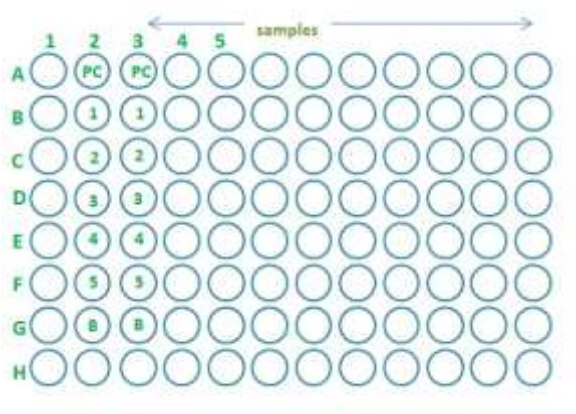
TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve with specific lot should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.159)
31.25	0.015
125	0.052
500	0.176
2000	0.592
8000	1.998

- Lot No.:
- Positive Control: refer to lot

Position of well



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 80 µl of standard dilutions, samples, or positive control to the well. Incubate for ***2 hours*** on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 80 µl Detection Antibody working solution to each well. Incubate for ***90 minutes*** on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 80 µl Streptavidin-HRP conjugate working solution to each well. Incubate for ***45 minutes*** on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 80 µl Substrate solution to each well. Incubate ***10 ~18 minutes*** on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 5 minutes.

The research samples were diluted by Dilution Buffer DB98. Its linearity and recovery was assayed by RCN2/Raptin Mouse ELISA Kit SK00168-12

Sample	Dilution Factor	Assayed (ng/mL)	Final (ng/mL)	Recovery (%)
Mouse Serum	1	0.645	0.645	100
Mouse Serum	2	0.326	0.651	100
Mouse EDTA Plasma	2	1.430	2.860	100
Mouse EDTA Plasma	4	0.772	3.089	109
Mouse EDTA Plasma	8	0.412	3.296	115
Rat EDTA Plasma	2	1.222	2.444	100
Rat EDTA Plasma	4	0.694	2.776	114
Rat Serun	4	0.918	3.674	100
Rat Serum	8	0.452	3.616	98

*Human Serum or EDTA Plasma was detectable by this ELISA Kit.

The New ELISA KITS for Metabolism Research (Research Use Only).

Biomarker Name	Catalog No.
Cholesin/C7ORF50 Human ELISA Kit	SK00027-06
Endotrophin (ETP) Human ELISA Kit	SK00009-06
Feimin/C5ORF24 Human ELISA Kit	SK00001-06
EPDR1 Human ELISA Kit	SK00023-06
GDF15 Human ELISA Kit	SK00108-01
Isthmin-1 (Ism1) Human ELISA Kit	SK00036-06
Human Sortilin ELISA Kit	SK00472-01
Human METRNL ELISA Kit	SK00478-06
Human Myonectin/CTRP15 ELISA Kit	SK00393-15
HS Soluble Neprilysin Human ELISA Kit	SK00724-01
High Sensitivity BDNF (H, R) ELISA Kit	SK00752-01
High Sensitivity Pro-BDNF (H) ELISA Kit	SK00752-09

More info check

- https://www.aviscerabioscience.net

References

1. Ling-Qi Xie, et al. Raptin, a sleep-induced hypothalamic hormone, suppresses appetite and obesity. Cell Research (2025) 35:165–185