

RETICULOCALBIN 2 (RCN2) /RAPTIN HUMAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
RCN2/RAPTIN CONCENTRATIONS IN HUMAN
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 HUMAN ELISA KIT
Catalog No.	SK00168-11
Lot No.	
Formulation	3 x 96 T
Standard range	62.5 - 16000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Animal free Cell Cultures
Dilution Factor	<i>(Optimal dilutions should be determined by each laboratory for each application)</i>
Specificity	Human and Mouse 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.	

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DESCRIPTION

The RCN2 gene (Reticulocalbin 2) encodes a calcium-binding protein primarily located in the lumen of the Endoplasmic Reticulum (ER). Raptin is a sleep-inducible hormone cleaved from the RCN2 protein. human Raptin is a peptide fragment of human RCN2 (28-249)¹. The Raptin is a circulating biomarker related to obesity.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal 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 a monoclonal antibody against RCN2.	168-11-01	1 plate
RCN2 Standard – 16 ng per vial of rh RCN2 (HEK293) in lyophilized.	168-11-02	1 vial
Detection Antibody Concentrate – 0.8 mL/vial, 10-fold concentrated of biotinylated antibody lyophilized.	168-11-03	vial
Positive Control - one vial of recombinant RCN2; lyophilized.	168-11-04	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.	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

Human Serum samples require 2- fold dilution by Dilution Buffer DB90.

Human EDTA plasma samples need 4~ 8 fold pre-dilution with Aviscera Bioscience's Dilution Buffer DB90.

A suggested 2-fold dilution is 40 µl sample per well + 40 µl Dilution Buffer DB90. A 4-fold dilution is 20 µl per well of sample + 60 µl per well of Dilution Buffer DB90. A 8-fold dilution is 10 µl per well of sample + 70 µl per well of Dilution Buffer DB90.

Human and Bovine shares over 90% similarity or identical in raptin sequence. Do not use fetal bovine serum, goat serum or other animal serum to pre-dilute the human samples.

Use animal free cell cultures for RCN2 assay.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

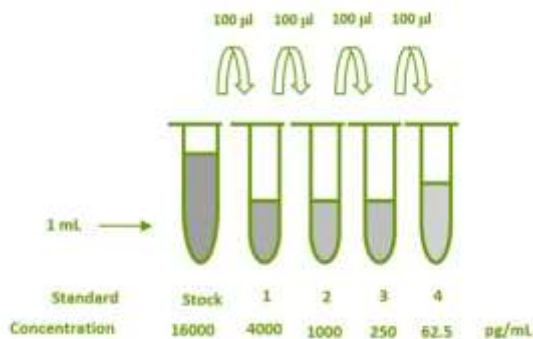
REAGENT PREPARATION

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 DB90. This reconstitution produces a stock solution of 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 #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **16000 pg/mL** standard (or refer to specific lot) serves as the high standard. The Dilution Buffer DB90 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 ng/ml
# 1	100 µl of stock	300 µl	4000 pg/mL
# 2	100 µl of 1	300 µl	1000 pg/ml
# 3	100 µl of 2	300 µl	250 pg/ml
# 4	100 µl of 3	300 µl	62.5 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 7.2 mL of **Antibody Diluent Solution (DB90)** into a 15 ml centrifuge tube and transfer 0.8 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 680 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 7.92 mL of **HRP Diluent solution (DB08C)** into a 15 mL centrifuge tube and transfer **80 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 680 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 80 µL per well of Dilution Buffer to Blank wells.
3. Add 80 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 80 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 80 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 80 µL of Substrate Solution to each well. Incubate for **20 ~25 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
Human RCN2 (28-249) His Tag (E. Coli)	100
Mouse RCN2 (31-252) His Tag (E. Coli)	100
Human RCN2 (250-317) His Tag (E. Coli)	0

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 (0.089)
62.5	0.030
250	0.099
1000	0.219
4000	0.428
16000	0.929

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

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 20 ~25 minutes on the plate shaker at RT. Protect from light.
↓
Add 80 µl Stop Solution to each well. Read at 450 nm within 5 minutes.

The 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 Nephilysin 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