HIGH SENSITIVITY EPDR1 (EPENDYMIN-RELATED PROTEIN 1) HUMAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN EPDR1 CONCENTRATIONS IN 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	HIGH SENSITIVITY EPDR1 HUMAN ELISA KIT
Catalog No.	SK00023-08
Lot No.	
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Cell Cultures
Dilution Factor	10~20 (***Optimal dilutions should be determined by each laboratory for each application)***
Specificity	Human EPDR1
Calibration	human EPDR1 recombinant (HEK293 derived)
Intra-assay Precision	2 - 6%
Inter-assay Precision	4 - 8%
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 CA 94089, USA
Tel: (408) 982 0300
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

Human Ependymin-related Protein 1 (EPDR1) is a new batokine secreted from adipose tissues and helps regulate energy balance and thermogenesis. Aviscera Bioscience Manufactured EPDR1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural EPDR1 from Serum and EDTA plasma samples in a sandwich ELISA format. The lysate of animal free HEK293 cells transfected with human EPDR1 cDNA can be detected by this ELISA Kit.

This immunoassay contains recombinant human EPDR1 derived from HEK293 animal free and high sensitivity antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural EPDR1 in human samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human EPDR1. The capture antibody can bind to the EPDR1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against EPDR1 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 EPDR1 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
EPDR1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against EPDR1.	**023-08-01**	**1 plate**
EPDR1 Standard – 12.8 ng per vial of rh EPDR1 (HEK293 derived) in a buffered protein base with preservative; lyophilized.	**023-08-02**	**1 vial**
Detection Antibody Concentrate – 1.5 mL/vial, 10-fold concentrated of biotinylated antibody against EPDR1 with preservative; lyophilized.	**023-08-03**	**1 vial**
Positive Control- one vial of recombinant rh EPDR1; lyophilized.	**023-08-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µl/vial, 125-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Sample Solution – 8 mL of buffered protein based solution with preservative.	**DB90**	**1 bottle**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB11C**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein 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 (250 – 300 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 or EDTA Plasma samples require pre-dilution by Dilution Buffer.

Human Serum samples or Plasma require 5 ~ 20 fold pre-dilution by Dilution Buffer.
A suggested 5-fold dilution is 20 µl sample per well + 80 µl Dilution Buffer per well. A suggested final 10-fold dilution is 10 µl per well of sample solution + 90 µl per well of Dilution Buffer. A suggested final 20-fold dilution is 5 µl per well of sample solution + 95 µl per well of Dilution Buffer.

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

For best recovery of samples assay, 50 µL per well of Sample Solution DB90 should be added by a 8-Channel Pipette to all assay wells prior to add diluted Sample Solution or Standard wells, Positive Control and Blank.

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.

EPDR1 Standard - Reconstitute the Human EPDR1 standard with 0.8 mL of Dilution Buffer. This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 3 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into the tube #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the high standard. The Dilution Buffer 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	0.8 ml	16000 pg/ml
# 1	40 µl of stock	600 µl	1000 pg/mL
# 2	300 µl of 1	300 µl	500 pg/ml
# 3	300 µl of 2	300 µl	250 pg/ml
# 4	300 µl of 3	300 µl	125 pg/ml
# 5	300 µl of 4	300 µl	62.5 pg/ml
# 6	300 µl of 5	300 µl	31.25 pg/ml
# 7	300 µl of 6	300 µl	15.6 pg/ml

Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare the 4-fold concentrated stock solution. Pipette 0.3 mL of Dilution Buffer into a 1.5 ml centrifuge tube and transfer 100 µL of 4-fold concentrated stock solution to prepare working solution. Discard the working solution of positive control after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.5 mL of **Antibody Diluent Solution (DB11C)** to produce a 10-fold concentrated stock solution prior to add 1x detection antibody solution to each well (Step 5).

For 96 wells test, **freshly** pipette 10.89 mL of **Antibody Diluent Solution (DB11C)** into a 15 ml centrifuge tube and transfer 1.1 mL of 10-fold concentrated stock solution to prepare working solution. *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 12.4 mL of **HRP Diluent solution (DB08C)** into a 15 mL centrifuge tube and transfer **100 µL** of **125-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 125-fold concentrated* Streptavidin *HRP ONLY at 2 -8°C for 12 months.*

ELISA PROTOCOL

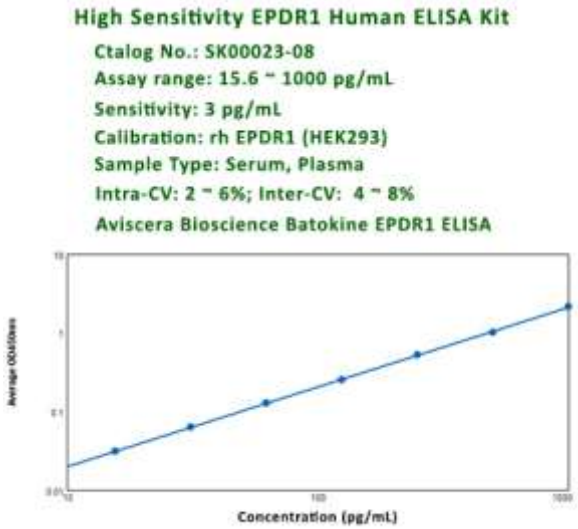
Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. **Add 50 µL per well of Sample Solution DB90 to all wells**. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, diluted 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 ***20~25 minutes*** on microplate shaker at room temperature. **Protect from light.**
10. Add 100 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 or 4-parameter fit to more accurately quantify the standard dilutions.

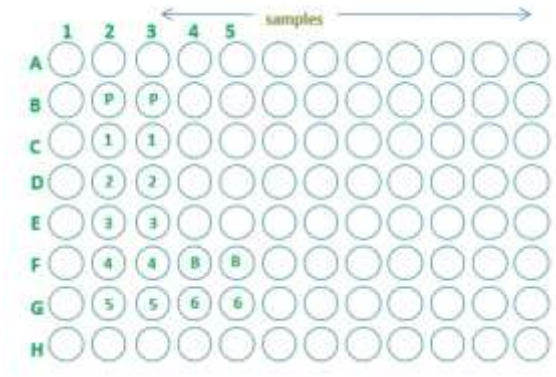
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human EPDR1 His Tag (HEK293 derived)	100
Human Adiponectin (HEK293 derived)	100
Human Irisin (HEK293 derived)	0
Human FNDC4 His Tag	0
Human COL3A1 C Terminal Globular Domain His Tag	0
Human Asprosin His Tag	0
Human COL4A1 C Terminal Globular Domain His Tag	0

Position of well



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 (0.109)
15.6	0.048
31.25	0.096
62.5	0.189
125	0.279
250	0.534
500	1.110
1000	2.102

- Lot No.:

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 50 µl per well of sample Solution to all assay wells. Add 100 µl of standard dilutions, diluted samples, or positive control to the well. Incubate for ***2 hours*** on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody working solution to each well. Incubate for ***90 minutes*** on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 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 100 µl Substrate solution to each well. Incubate ***20~25 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.

Human Sortilin ELISA Kit	SK00472-01
Human Lipocalin 13 ELISA Kit	SK00648-06
Human METRNL ELISA Kit	SK00478-06
Human Myonectin/CTRP15 ELISA Kit	SK00393-15
HS Soluble Neprilysin Human ELISA Kit	SK00724-01
Human Soluble LOX-1 ELISA Kit	SK00006-01
Human CHGA (19-131)/Vasostatin-2 ELISA Kit	SK00084-02

More info check

- https://www.aviscerabioscience.net

The reliable research Biomarker ELISA KITS were manufactured by Aviscera Bioscience for Research Use Only.

Biomarker Name	Catalog No.
Cholesin/C7ORF50 Human ELISA Kit	SK00027-09
Feimin/C5OEF24 Human ELISA Kit	SK00001-06
FAM19A1/TAFA1 Human ELISA Kit	SK00419-06
EPDR1 Human ELISA Kit	SK00023-06
LPGDS Human ELISA Kit	SK00025-06
Human Endotrophin ELISA Kit	SK00009-08
Nidogen-2 Human ELISA Kit	SK00480-06
Isthmin-1 Human ELISA Kit	SK00036-06
Human Irisin ELISA Kit	SK00170-08
HS BDNF (Human, Rat) ELISA Kit	SK00752-01