HIGH SENSITIVITY ENDOTROPHIN (ETP) HUMAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ENDOTROPHIN 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 ENDOTROPHIN (ETP) HUMAN ELISA KIT**
Catalog No.	SK00009-08
Lot No.	20115048
Formulation	96 T
Standard range	7.8 - 500 pg/mL
Sensitivity	1.5 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma, Cell Cultures
Dilution Factor	200~400 (***Optimal dilutions should be determined by each laboratory for each application)***
Specificity	Human Endotrophin
Calibration	human Endotrophin recombinant (HEK293 derived)
Intra-assay Precision	2 - 5%
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.	

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DESCRIPTION

Human Endotrophin (ETP) is a bioactive peptide fragment (3101-3177) derived from the C Terminal Globular Domain 5 of human Collagen VIα3 (COL6A3). The researchers reported human Endotrophin is a novel circulating biomarker related to cardiovascular disease (CAD and Heart Failure), obesity, insulin resistance, fibrosis, inflammation and kidney injury. Aviscera Bioscience Manufactured High Sensitivity Human Endotrophin (ETP) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Endotrophin from Serum and EDTA plasma samples in a sandwich ELISA format. The lysate of animal free HEK293 cells transfected with human endotrophin cDNA can be detected by this ELISA Kit.

This immunoassay contains recombinant human Endotrophin 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 Endotrophin in human samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for human Endotrophin. The capture antibody can bind to the Endotrophin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Endotrophin 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 Endotrophin 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
Endotrophin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against ETP.	**009-08-01**	**1 plate**
Endotrophin Standard – 8 ng per vial of rh Endotrophin (HEK293 derived) in a buffered protein base with preservative; lyophilized.	**009-08-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against ETP with preservative; lyophilized.	**009-08-03**	**1 vial**
Positive Control- one vial of recombinant BDNF; lyophilized.	**009-08-04**	**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 protein based solution with preservative.	**DB01**	**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.	**DB08B**	**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.

If the level of Endotrophin in samples is 10 ~ 80 ng/mL. Human Serum or EDTA Plasma samples require 200 ~ 400 fold pre-dilution by Dilution Buffer.
A suggested 20-fold dilution is 10 µl sample + 190 µl Dilution Buffer. A suggested final 200-fold dilution is 10 µl per well of 20 –fold diluted sample solution + 90 µl per well of Dilution Buffer. A suggested final 400-fold dilution is 5 µl per well of 20 –fold diluted sample solution + 95 µl per well of Dilution Buffer.
If the level of Endotrophin in samples is higher than 80 ng/mL, may require more fold pre-dilution.

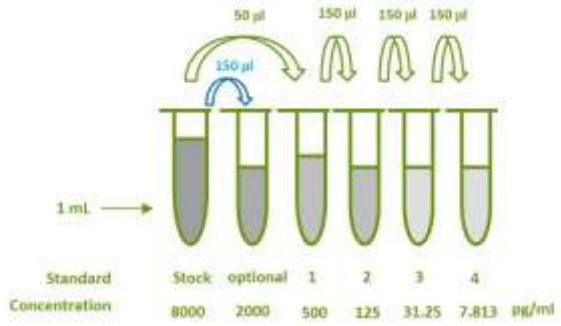
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

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.

Endotrophin Standard - Reconstitute the Human Endotrophin standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer into the tube #2 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **500 pg/mL** standard (or refer to specific lot) 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. Optional set 2000 pg/mL of standard as the highest standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	8000 pg/ml
optional	150 µl of stock	450 µl	2000 pg/mL
# 1	50 µl of stock	750 µl	500 pg/ml
# 2	150 µl of 1	450 µl	125 pg/ml
# 3	150 µl of 2	450 µl	31.25 pg/ml
# 4	150 µl of 3	450 µl	7.813 pg/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer 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 1.2 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 9.45 mL of **Antibody Diluent Solution (DB11C)** into a 15 ml centrifuge tube and transfer 1.05 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 11.828 mL of **HRP Diluent solution (DB08B)** 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 ***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 Endotrophin His Tag (HEK293 derived)	100
Human Endotrophin (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

The mouse or rat pooled research serum or EDTA plasma samples were detected by this elisa kit less than 20 pg/mL. Its serial dilution with dilution buffer does not have any linearity recovery. The data indicated the mouse or rat serum or plasma can not be detected by this ELISA Kit.

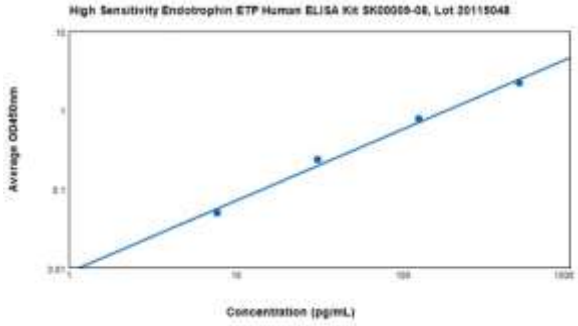
TYPICAL STANDARD CURVE

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

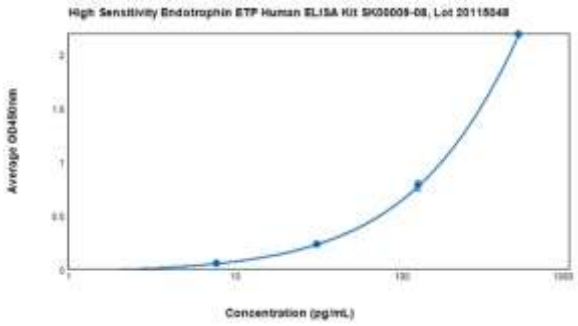
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.069)
7.813	0.049
31.25	0.231
125	0.775
500	2.050
2000 (optional)	2.739

- Lot No.: 20115048
- Positive Control: 20 ~ 160 pg/mL

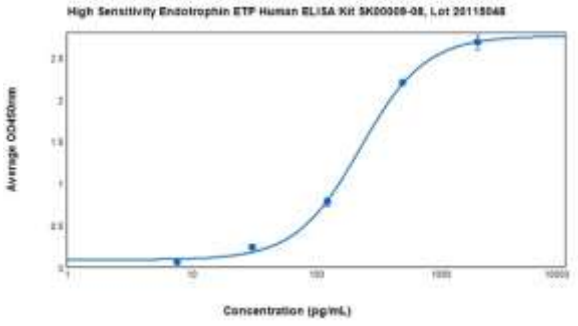
Standard Curve (7.813 ~ 500 pg/mL) was fited by Log-Log:



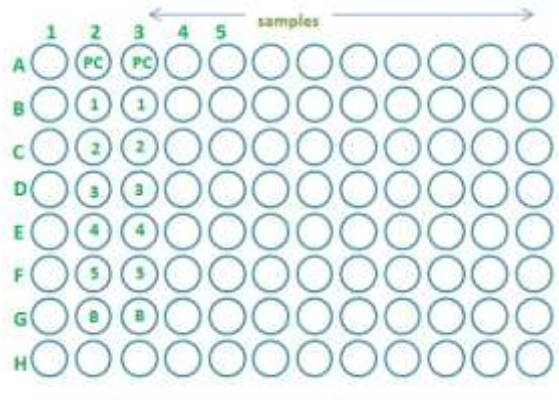
Standard Curve (7.813 ~ 500 pg/mL) was fited by 4-parameter:



Optional Standard Curve (7.813 ~ 2000 pg/mL) was fited by 4-parameter:



Position of well



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 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 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.

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

Biomarker Name	Catalog No.
Cholesin/C7ORF50 Human ELISA Kit	SK00027-06
Feimin/C5ORF24 Human ELISA Kit	SK00001-06
FAM19A1/TAFA1 Human ELISA Kit	SK00419-06
EPDR1 Human ELISA Kit	SK00023-06
Soluble NOTCH3 NT Human ELISA Kit	SK00002-11
LPGDS Human ELISA Kit	SK00025-06
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
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