HUMAN LIPOCALIN 13/OBP2A ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN LIPOCALIN 13 CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**LIPOCALIN 13 OBP2A ELISA KIT HUMAN**
Catalog No.	SK00648-08
Lot No.	20114830
Formulation	96 T
Standard Range	0.195 ~ 25 ng/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	**2 ~ 4 (Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Human
Calibration	Human Lipocalin 13 recombinant
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 8%
Storage	2 - 8° C for 6 months, see page 2~3 for detail
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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

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

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

This Human Lipocalin 13 OBP2A ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Lipocalin 13 from human serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant Human Lipocalin 13 OBP2A and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Lipocalin 13 in mouse samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for Human Lipocalin 13 OBP2A. The capture antibody can bind to the Human Lipocalin 13 OBP2A in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against Human Lipocalin 13 OBP2A 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 Human Lipocalin 13 OBP2A 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

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_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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_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
Lipocalin 13 Microplate - 96 well polystyrene microplate coated with a monoclonal antibody specific for Human Lipocalin 13 OBP2A.	**648-08-01**	**1 plate**
Lipocalin 13 Standard – 200 ng/vial of lyophilized recombinant Human Lipocalin 13 OBP2A.	**648-08-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated monoclonal antibody against Human Lipocalin 13 OBP2A.	**648-08-03**	**1 vial**
Positive Control Concentrate– one vial of lyophilized recombinant Human Lipocalin 13 OBP2A.	**648-08-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µl of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**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.	**DB41**	**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**
Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 6 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored

at -20° C. Streptavidin-HRP Conjugate and 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 Order Code: 00700-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Human serum and plasma samples may require a 2-4 fold dilution. A suggested 2-fold dilution is 50 µl of sample per well + 50 µl Dilution Buffer per well. A suggested 4-fold dilution is 25µl of sample per well + 75 µl Dilution Buffer per well.

Rat serum and plasma samples DO NOT require dilution.

Optimal dilutions should be determined by each laboratory for each application.

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.

Human Lipocalin 13 Standard - Reconstitute the Lipocalin 13 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **25 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Stored the stock solution at -70 °C for a few days.

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	200 ng/ml
# 1	100 µl of stock	700 µl	25 ng/ml
# 2	250 µl of 1	250 µl	12.5 ng/ml
# 3	250 µl of 2	250 µl	6.25 ng/ml
# 4	250 µl of 3	250 µl	3.125 ng/ml
# 5	250 µl of 4	250 µl	1.56 ng/ml
# 6	250 µl of 5	250 µl	0.78 ng/ml
# 7	250 µl of 6	250 µl	0.39 ng/ml
# 8	250 µl of 7	250 µl	0.195 ng/ml

Positive Control Concentrate- Reconstitute the Positive Control with 1.0 mL of Dilution Buffer to produce 1x working solution. Discard the 1x working solution after use.

Detection Antibody – Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB41)** to produce a 10-fold concentrated solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. For the 96 well test, freshly Pipette 9.45 mL of **Antibody Diluent Solution (DB41)** into a 15mL centrifuge tube and transfer 1.05 mL of 10-fold

concentrated solution to prepare 1x working solution. For partial strip test, prepare 900 µl per strip (8-well) of working solution. Store the stock solution at -20 °C for a few days.

Streptavidin-HRP Conjugate – For 96 wells test, freshly Pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare 1x working solution **(protect from light)**. 1x working solution should be used in 10-20 min.

For partial strip test, prepare 900 µl per strip (8-well) of working solution. Store the stock solution at 2 ~ 8 °C for 10 months. **Do Not Freeze.**

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 of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions, 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 per well of 1x Detection Antibody working solution. Cover with plate sealer and incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µl per well of 1x working solution of Streptavidin-HRP Conjugate. Cover with plate sealer and 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 10-15 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 4-parameter 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 Lipocalin 13 OBP2A	100
Mouse Lipocalin 13	0
Rat Lipocalin 2	0
Human Lipocalin 2	0

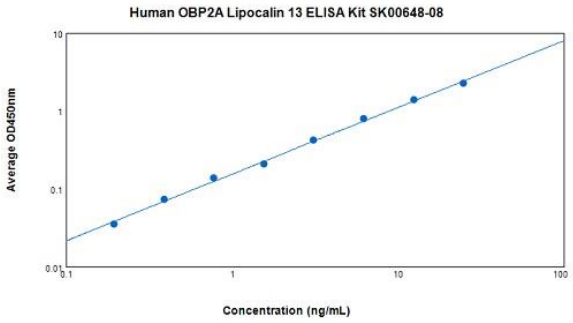
TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.109)
0.195	0.035
0.39	0.072
0.78	0.136
1.56	0.209
3.125	0.418
6.25	0.789
12.5	1.394
25	2.239

- Lot No.: 20114830
- Positive Control (1X solution): 5 – 20 ng/mL

Standard Curve by log-log-fit



Research Sample Test:

The research samples were diluted by Dilution Buffer DB01. Its linearity and recovery was assayed by Human OBP2A Lipocalin 13 ELISA Kit SK00648-08

Sample Type	Dilution Factor	Assayed (ng/mL)	Final (ng/mL)	Recovery (%)
Human EDTA Plasma A	2 X	23.284	46.569	100
Human EDTA Plasma A	4 X	11.370	45.481	102
Human EDTA Plasma A	8 X	5.410	43.280	93
Human Serum B	1 X	8.928	8.928	100
Human Serum B	2 X	4.419	8.837	98.9
Human Serum B	4 X	2.135	8.541	95.6

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody working solution. Incubate 90 minutes on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well of 1x Streptavidin-HRP conjugate working solution. Incubate 45 minutes on microplate shaker at RT. **Protect fom light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-15 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 5 min.