HIGH SENSITIVITY HUMAN LEAP-2 (38-77) PEPTIDE ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN LEAP-2 (38-77) PEPTIDE CONCENTRATIONS IN SERUM AND EDTA PLASMA

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

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

PURCHASE INFORMATION:

ELISA NAME	**HIGH SENSITIVITY HUMAN LEAP-2 (38-77) PEPTIDE ELISA KIT**
Catalog No.	**SK00007-06**
Lot No.	
Formulation	**96 T**
Standard range	**0.064- 40 ng/mL**
Dynamic range	**0.064 – 8 ng/mL**
Sensitivity	**~0.02 ng/mL**
Luminescence Read	**On Top**
Sample Volume	**40 µL**
Extraction	**Require Peptides Extracted from Serum, EDTA Plasma or Tissue Homogenates**
Sample Type	**Serum, EDTA Plasma**
Specificity	**Human, Mouse**
Intra-assay Precision	**2-6%**
Inter-assay Precision	**4-9%**
Storage	**2-8°C for 8 months. For longer storage, See page 3 for detail**

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INTRODUCTION

Liver-expressed antimicrobial peptide 2 (LEAP2) is a 40-amino-acid hepatokine and cationic antimicrobial peptide that acts as an endogenous antagonist / inverse agonist of the growth hormone secretagogue receptor (GHSR). It regulates energy homeostasis, appetite, and glucose metabolism by inhibiting ghrelin-mediated signaling. LEAP2 levels increase in obesity and decrease in energy-deficit states.

High Sensitivity Human LEAP-2 (38-77) Peptide ELISA employs the quantitatively competitive enzyme immunoassay technique in which Human LEAP-2 (38-77) Peptide present in samples compete with a fixed amount of biotinylated Human LEAP-2 (38-77) Peptide for sites on purified rabbit IgG specific against Human LEAP-2 (38-77) Peptide. During the incubation period, the rabbit IgG specific for Human LEAP-2 (38-77) Peptide binds to the goat anti-rabbit IgG pre-coated onto the microplate. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a mixed chemiluminescence substrate solution is added to the wells. The enzyme reaction yields luminescence light was read on top. The intensity of the luminescence light measured is in inverse proportion to the amount of Irisin bound in the initial step. The sample values are then read off the standard curve.

High Sensitivity Human LEAP-2 (38-77) Peptide ELISA has been shown to accurately quantify the Human LEAP-2 (38-77) Peptide and natural Human LEAP-2 (38-77) Peptide. Results obtained using natural Irisin showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_Some vials contain small quantities of material, therefore centrifuge before use.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well microplate white pre-coated with polyclonal anti rabbit IgG Fc.	**RM01-WP**	**1 plate**
Human LEAP-2 (38-77) Peptide Standard – 40 ng/vial in a buffered protein; lyophilized.	**007-06-01**	**1 vial**
LEAP-2 (38-77) Peptide Biotin – 0.5 mL of 10-fold concentrated with preservative; lyophilized.	**007-06-02**	**1 bottle**
LEAP-2 (38-77) Antibody – 0.5 mL of 10-fold concentrated rabbit purified IgG against LEAP-2; lyophilized.	**007-06-03**	**1 bottle**
Positive Control – one vial of Peptide ; lyophilized (optional).	**007-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 30 mL of Blue Color. Ready to use.	**DB01B**	**1 bottle**
Antibody Diluent Solution – 8 mL of Yellow Color solution. Ready to use.	**DB01Y**	**1 bottle**
Biotin Diluent Solution – 8 mL of Red Color solution. Ready to use.	**DB01R**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative. Ready to use.	**DB08C**	**1 bottle**
Wash Buffer - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Chemiluminescence Substrate Solution A- 5	**CLSA01**	**1 bottle**

mL solution in amber bottle		
Chemiluminescence Substrate Solution B- 5 mL solution in clear bottle.	**CLSB01**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Antibody and Biotin, Dilution Buffer and HRP Diluent Solution should be stored at -20 °C.
Do not use kit past expiration date.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 12 months at 2 – 8 °C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring luminescence light on top of plate.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 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) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples require peptides extraction from human serum, EDTA plasma, cell cultures and or tissue homogenates by C18 affinity column. Refer to follow Peptide Extraction Protocol.

Optimal dilutions should be determined by each laboratory for each application with a 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 into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

LEAP-2 (38-77) Peptide Standard - Reconstitute the **standard** with 1.0 mL of The **Blue Color Dilution Buffer (DB01B)**. This reconstitution produces a stock solution of 40 ng/mL. Gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a 5-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **40 ng/mL** standard serves as the high standard. The **Blue Color Dilution Buffer (DB01B)** serves as the zero standard (0 ng/mL) which was named as total binding. Store the stock solution at -20 ~ -70°C.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0mL	40 ng/ml
# 1	50µl of stock	200µl	8 ng/ml
# 2	50µl of 1	200µl	1.6 ng/ml
# 3	50µl of 2	200µl	0.32 ng/ml
# 4	50µl of 3	200µl	0.064 ng/ml
Total Binding	0	250µl	0 ng/mL

The 40 µL per well of the Blue Colored Standard solution, Positive Control and extracted samples was added into each well.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of The **Blue Color Dilution Buffer (DB01B)**. **Note:** After use discard the Positive Control . It is for one time use only.

Detection Antibody – Reconstitute the **LEAP-2 (38-77) Antibody** with **0.5 mL** of **Yellow Color Antibody Diluent Solution DB01Y** to produce a 10 -fold concentrated stock solution. For 96 wells test, Freshly Pipette 4.5 mL of **Yellow Color Antibody Diluent Solution DB01Y** into a 15 mL centrifuge tube and transfer 0.5 mL of 10-fold concentrated stock solution to prepare 1x Antibody working solution. *For partial strips test, Freshly prepare 0.35 mL per strip (8-well) of 1x Antibody working solution. Store the stock solution at* -20 °C ~ -70 °C for 2 ~ 3 days. **The 40 µL per well of the Yellow Colored 1 x antibody working solution was added into each well.**

Biotin Tracer - Reconstitute the **LEAP-2 Biotin** with 0.5 mL of The **Red Color Biotin Diluent Solution (DB01R)** to produce a 10 -fold concentrated stock solution. For 96 wells test, Freshly Pipette 4.5 mL of **Red Color Biotin Diluent Solution (DB01R)** into a 15 mL centrifuge tube and transfer 0.5 mL of 10-fold concentrated stock solution to prepare 1x Biotin working solution. *For partial strips test, Freshly prepare 0.35 mL per strip (8-well) of 1x Biotin working solution. Store the stock solution at* -20 °C ~ -70 °C for 2 ~ 3 days. **The 40 µL per well of the Red Color 1 x biotin working solution was added into each well.**

Streptavidin-HRP Conjugate – For 96 wells test, freshly Transfer 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB08C)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within 10-20 minutes **(protect from light)**. *For partial strips test, Freshly prepare 0.9 mL per strip (8-well) of 1x working solution. Store the stock solution at* 2~8 °C for 12 months.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave wells G4, G5 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set A2, A3 as total binding (TB). Add 40 µL per well of **Blue Color Dilution Buffer (DB01B)**.
5. Add 40 µL per well of the **Blue Color standard solutions** from #4 to #S (reverse order of serial dilution) to the appropriate wells (B2, B3 to H2, H3). Add 40 µL per well of The Blue Color **Positive Control** into wells F4, F5. Add 50 µL per well of pre-diluted peptide extracted **samples** into appropriate wells.
6. Add 40 µL per well of **Yellow Colored 1x Antibody Solution** into total binding, standard, positive control and sample wells. All the well of each standard, Positive control and or diluted sample will be changed to light **Green Color**. Cover with plate sealer and incubate on microplate shaker (200-300rpm) at room temperature for 2 hours. **NOTE: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.**
7. Add 40 µL per well of **Red Colored 1x Biotin**

Solution into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (200-300rpm) at room temperature for 2 hours. **NOTE: DO NOT ADD Biotin Solution to Blank wells.**

8. Aspirate wells and wash 4 times with 300 µL of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well, including blanks. Incubate on microplate shaker (200-300rpm) for 45 minutes at room temperature. **Protect from light**.
10. Aspirate and wash as step 8.
11. Add 100 µL of mixed **Chemiluminescence Substrate Solution** to each well. Incubate on microplate shaker (200-300rpm) for 2~3 minutes at room temperature. **Protect from light**. Read the luminescence light by Luminescence Read on the Top of whit plate at 5 min or 10 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D. absorbances. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 8~40 ng/mL may result in inaccurate, low LEAP-2 levels. Such samples require further external predilution according to expected LEAP-2 values with Dilution Buffer in order to precisely quantify the actual Irisin level. **Optimal dilutions should be determined by each laboratory for each application.**

CALIBRATION

This immunoassay is calibrated against a highly purified Human LEAP-2 (38-77) Peptide.

SENSITIVITY

~0.02 ng/mL

TYPICAL DATA

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

LAYOUT	STANDARD (NG/ML)	RLU (CORRECTED)
BLANK		0 (9696)
STOCK STD	40	13974
STD1	8	27159
STD2	1.6	43160
STD3	0.32	82031
STD4	0.064	95419
TOTAL BINDING	0	125122

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

SPECIFICITY

This assay recognizes both natural and recombinant Irisin. The factors listed below were prepared at 500 µg/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY (%)
Human LEAP-2 (38-77)	100
Human Ghrelin	0
Human Irisin	0
Human Endotrophin	0
Human Raptin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 40 µL of the **Blue Color** standard, extracted samples, positive control to the well. Add 40 µL of **Yellow Color** **1x Antibody Solution** to each well used, except blanks. Incubate 2 hours on the plate shaker at RT. **DO NOT ASPIRATE AND WASH PLATE. PROCEED TO NEXT STEP.**
⬇
Add 40 µL of **Red Color** **1x Biotin Solution** to each well used, except blanks. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL Streptavidin-HRP conjugate working solution to all wells, including blanks. Incubate 45 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µL mixed Chemiluminescence Substrate Solution to each well. Incubate 2~ 3 min on plate shaker at RT. **Protect from light.**
⬇
Read Luminescence light by Luminescence Read on top of white plate at 5 min or 10 min.