Antibody and Immunoassay Services

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Human lipocalin-13 Immunoassay Kit

Catalogue Number: 31360

For the quantitative determination of human lipocalin-13 concentration
in serum and plasma samples.

This package insert must be read in its entirety before using this product.
Use only the current version of product data sheet enclosed with the kit.

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Version: 1.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INSTRUCTION

Lipocalin-13 (LCN13), also known as odorant binding protein 2A (Obp2a), is one newly identified potential candidate to regulate glucose and lipid metabolism. LCN13 is mainly found in liver, skeletal muscle and the pancreas out of multiple tissues in mice. Animal studies suggest LCN13 protein protects against hepatic steatosis.[1] It was found that LCN13 can attenuate hyperglycemia and insulin resistance in both insulin-dependent and independent manners.[2] Expression of LCN13 was found down-regulated in the liver and circulation of diet induced obese, genetic obese (ob/ob) and diabetic (db/db) mouse models[3]. LCN13 is evolutionary conserved in many species including human. Due to its potent effects demonstrated by recent studies, it is suggested to be a promising drug target to treat obesity and T2DM.[2]

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich ELISA. The immunoplate is pre-coated with a rabbit polyclonal antibody specific for human lipocalin-13. Standards and samples are pipetted into the wells and any human lipocalin-13 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin labelled polyclonal antibody specific for human lipocalin-13 is added to the wells. After wash step to remove any unbound reagents, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human lipocalin-13 bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human lipocalin-13, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human lipocalin-13 ELISA kit is designed for quantification of human lipocalin-13 in serum sample.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a polyclonal antibody against human lipocalin-13, sealed.
2. 10×Wash buffer-50 ml.
3. 5×Assay buffer-20 ml.
4. 100×Detection antibody solution-A biotin labelled polyclonal antibody against human lipocalin-13, 0.12 ml.
5. Human lipocalin-13 standard-200 pg of recombinant human lipocalin-13 in a buffered protein base, lyophilised.
6. 100×STP-HRP solution-0.12 ml.
7. Substrate solution- 12 ml, ready for use.
8. Stop solution-12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Horizontal micro-plate shaker capable of 300 rpm.

STORAGE

The kit should be stored at 2-8℃ upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human lipocalin-13 microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8℃ for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

1. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 ml) with 80 ml of distilled water or deionized water. If precipitates are observed in the 5× Assay buffer bottle, warm the bottle in a 37℃ water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8℃ for up to one month.

2. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 ml) with 450 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37℃ water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8℃ for up to one month.

3. **1×Detection antibody solution**.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8℃ immediately after the necessary volume is removed.

4. 1×STP-HRP solution.

Spin down the 100×STP-HRP solution briefly and dilute the desired amount of the 100×STP-HRP solution 1:100 with 1×Assay buffer, 100 µl of the 1× STP-HRP solution is required per well. Prepare only as much 1× STP-HRP solution as needed. Return the 100×STP-HRP solution to 2-8℃ immediately after the necessary volume is removed.

PREPARATION OF STANDRADS AND SAMPLES

Human lipocalin-13 standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 200 pg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard Volume	Volume of 1×Assay buffer	Concentration
200 pg/ml stock	-	200 pg/ml
250 µl of 200 pg/ml	250 µl	100 pg/ml
250 µl of 100 pg/ml	250 µl	50 pg/ml
250 µl of 50 pg/ml	250 µl	25 pg/ml
250 µl of 25 pg/ml	250 µl	12.5 pg/ml
250 µl of 12.5 pg/ml	250 µl	6.25 pg/ml
250 µl of 6.25 pg/ml	250 µl	3.12 pg/ml

1x Assay buffer serves as the zero standard (0 ng/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -20℃ for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 4-fold dilution in 1×Assay buffer. A suggested dilution step is to add 100 µl of sample to 300 µl of 1×Assay buffer.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µl of standard or sample per well. Seal the plate with a plate cover. Incubate at room temperature for 2 hour, shaking the plate at 300 rpm on a horizontal micro-plate shaker.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µl of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

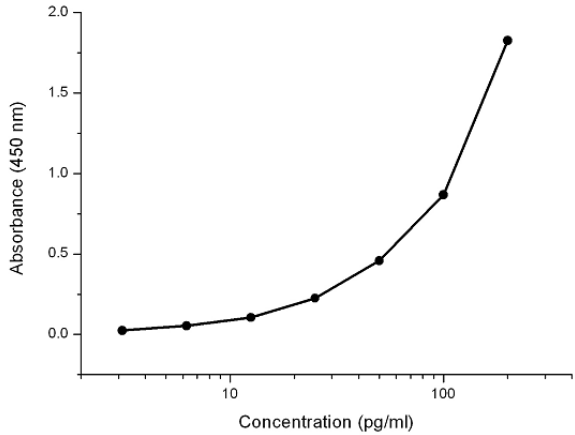
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human lipocalin-13 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human lipocalin-13 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Lipocalin-13 (pg/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.069	0
3.12	0.093	0.024
6.25	0.122	0.053
12.5	0.174	0.105
25	0.294	0.225
50	0.527	0.458
100	0.937	0.868
200	1.895	1.826

Human lipocalin-13 standard curve



ASSAY CHARACTERISTICS

A. Sensitivity:

The lowest level of human LCN13 that can be detected by this assay is 3.12 pg/ml.

B. Specificity:

Cross Reactivity of recombinant proteins

Analyte	Cross Reactivity
Human FABP4	No
Human LCN2	No
Human Adiponectin	No
Human FGF21	No

C. Precision:

Intra-assay Precision (Precision within an assay)
Two samples of known concentration were tested 12 times on one plate.

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	52.95	1.46	2.8
2	32.21	1.30	4.0

Inter-assay Precision (Precision between assays)
Two samples of known concentration were tested in 10 separate assays.

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	51.13	3.48	6.8
2	43.59	2.42	5.5

E. Linearity:

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human LCN13 were serially diluted with the 1 ×Assay buffer to produce samples with values within the dynamic range of the assay.

Sample 1

Dilution	Measured (pg/ml)	Expected (pg/ml)	Recovery (%)
1/2	54.52	54.52	100
1/4	29.745	27.26	109
1/8	13.18	13.63	97

Sample 2

Dilution	Measured (pg/ml)	Expected (pg/ml)	Recovery (%)
1/2	59.96	59.96	100
1/4	31.835	29.98	106
1/8	16.8975	14.99	113

REFERENCES:

[1] Lipocalin 13 protein protects against hepatic steatosis by both inhibiting lipogenesis and stimulating fatty acid β-oxidation. Sheng L, Cho KW, Zhou Y, Shen H, Rui L. *J Biol Chem.* 2011 Nov 4;286(44):

[2]. Lipocalin-13 regulates glucose metabolism by both insulin-dependent and insulin-independent mechanisms. Cho KW, Zhou Y, Sheng L, Rui L. *Mol Cell Biol.* 2011 Feb;31(3):450-7.

[3]. Lipocalin 13 regulation of glucose and lipid metabolism in obesity. Zhou Y, Rui L. *Vitam Horm.* 2013;91:369-83.

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well four times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation