ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Lipocalin-2 ELISA Kit

(Catalog Number: 31050)

For the quantitative determination of human lipocalin-2 concentrations in serum, plasma, urine or cell culture supernate samples

IMD (Hong Kong)

Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)

Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

Version 2.0

TABLE OF CONTENTS

Contents	Page
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	1
INTENDED USE	1
REAGENTS SUPPLIED	1
OTHER MATERIALS REQUIRED, BUT NOT PROVIDED	1
STORAGE	2
PREPARATION OF REAGENTS	2
PREPARATION OF STANDARDS AND SAMPLES	2
ASSAY PROCEDURE	3
CALCULATION	3
TYPICAL STANDARD CURVE	3
ASSAY CHARACTERISTICS	4
REFERENCES	4
SUMMARY OF ASSAY PROCEDURE	5

INTRODUCTION

Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), 24p3, or neutrophil lipocalin (NL), is a 25-kDa secretory glycoprotein[1]. LCN2 has been implicated in a variety of cellular processes including the innate immune response, differentiation, tumorigenesis, and cell survival[2,3]. It appears to be upregulated in various inflammation and infection conditions. Several reports suggest that LCN2 may represent a sensitive biomarker for various renal injuries[4] and is associated with several types of cancers, including breast cancer[5], ovarian, colorectal, and pancreatic cancers[6,7]. Furthermore, a growing body of evidence suggests that serum levels of lipocalin-2 are correlated with obesity, insulin resistance, hyperglycemia, coronary heart disease and fatty liver disease in humans[8-11].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against human LCN2. The microtiter plate is pre-coated with a monoclonal antibody specific for human LCN2. Standards and samples are added into the wells and any human LCN2 present is captured by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked monoclonal antibody specific for human LCN2 is added to the wells. After a final washing step to remove any unbound reagents, a HRP substrate solution is added and color develops in proportion to the amount of human LCN2 bound initially. The reaction is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human LCN2, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human LCN2 ELISA kit is designed for quantification of human lipocalin-2 in serum, plasma, urine and cell culture supernate samples.

REAGENTS SUPPLIED

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

1. Microtiter Strips (96 wells), coated with a mouse monoclonal antibody against human LCN2
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 20 mL
4. 100×Detection antibody solution, a mouse monoclonal antibody against human LCN2 conjugated to horseradish peroxidase, 0.12 mL
5. Human LCN2 standard, 25 ng of recombinant human LCN2, lyophilized
6. Substrate solution, 12 mL, ready for use
7. 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

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human LCN2 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored for up to one month at 2-8°C.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 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°C water bath until the precipitates disappear. 1×Assay buffer may be stored for up to one month at 2-8°C.

B. 1×Wash buffer

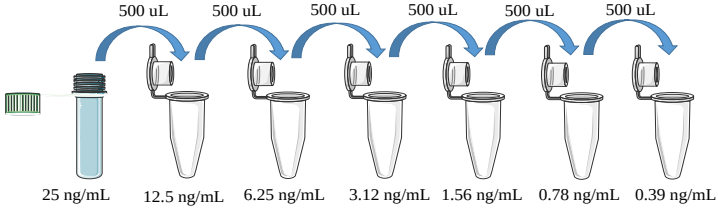
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Wash buffer may be stored for up to one month at 2-8°C.

C. 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°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human LCN2 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 25 ng/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 shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and frozen at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **10-fold** dilution in 1×Assay buffer. A suggested dilution step is to add 30 µL of sample to 270 µL of 1×Assay buffer. Dilution factors of urine and cell culture supernate samples need to be optimized by the user. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

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

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
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 4 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µL of Stop solution to each well, mix well by gently tapping the plate.
7. 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 LCN2 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 LCN2 concentration of samples from standard curve and multiply the value by the dilution factor.

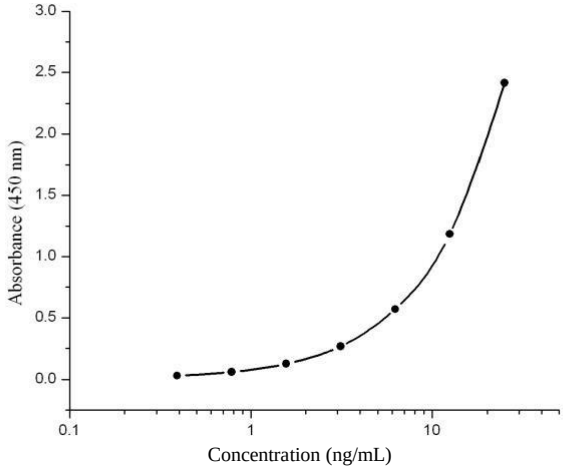
TYPICAL STANDARD CURVE

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

Human LCN2 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.076	0
0.39	0.107	0.031
0.78	0.137	0.061

1.56	0.203	0.127
3.12	0.343	0.267
6.25	0.647	0.571
12.5	1.26	1.184
25	2.492	2.416

Human lipocalin-2 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of LCN2 that can be detected by this assay is 0.39 ng/mL.

B. Specificity

The antibody pair used in this assay is specific to human LCN2 and does not cross-react with mouse and rat LCN2, and other cytokine or hormone molecules.

REFERENCES

1. Kjeldsen, L. et al. (1993) *J. Biol. Chem.* 268:10425-32
2. Yang, J. et al. (2002) *Mol. Cell.* 10:1045-56.
3. Flo, T.H. et al. (2004) *Nature* 432:917-21.
4. Mishra, J. et al. (2005) *Lancet* 365:1231-6.
5. Yang, J. et al. (2009) *Proc Natl Acad Sci U S A 106(10):3913-8.*
6. Hanai, J. et al. (2005) *J. Biol. Chem.* 280:13641-7.
7. Lee, H.J. et al. (2005) *Int. J. Cancer* 118:2490-7.
8. Wang Y, et al. (2007) *Clin Chem* 53(1):34-41.

9. Yan QW, et al. (2007) *Diabetes* 56(10):2533-40.
10. Ruby LC Hoo, et al. (2008) *Expert Rev. Endocrinol Metal* 3(1):29-41.
11. Milner KL, et al. *Hepatology* [Epub ahead of print]

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample per 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 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