



Mouse Lipocalin-2 Immunoassay Kit

Catalogue Number: 32050

For the quantitative determination of mouse lipocalin-2 concentrations
in serum, plasma and cell culture supernate 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.

Version: 3.0

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

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INTRODUCTION

Lipocalin-2(LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), 24p3, or neutrophil lipocalin (NL), is a 25-kDa secretory glycoprotein¹. LCN2 has been implicated in a variety of cellular processes including the innate immune response, differentiation, tumorigenesis and cell survival²⁻³. It appears to be up-regulated in various inflammation and infection conditions. Several reports suggest that LCN2 may represent a sensitive biomarker for various renal injuries⁴ and is associated with several types of cancers, including breast cancer⁵, ovarian, colorectal and pancreatic cancers⁶⁻⁷. Furthermore, a growing body of evidence suggests that serum levels of LCN2 are correlated with obesity, insulin resistance, hyperglycemia, coronary heart disease and fatty liver disease in humans⁸⁻¹¹.

PRINCIPLE OF THE ASSAY

This assay is a sandwich ELISA using antigen-affinity-purified polyclonal antibodies against mouse LCN2. The immunoplate is pre-coated with anti-mouse LCN2 capture antibody. Standards and samples are added to the wells and any mouse LCN2 present is captured by the immobilized antibody. After wash step to remove any unbound substances, a biotin labelled anti-mouse LCN2 detection antibody is added. After washing procedure, 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 mouse LCN2 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 mouse LCN2, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This mouse LCN2 ELISA kit is designed for quantification of mouse LCN2 in serum, plasma and cell culture supernate samples.

REAGENTS SUPPLIED

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

1. Micro-titre strips (96 wells)-Coated with anti-mouse LCN2 polyclonal antibody, sealed.
2. 10×Wash buffer-50 ml.
3. 5×Assay buffer-30 ml.
4. 100×Detection antibody solution-A biotin labelled polyclonal antibody against mouse LCN2, 0.12 ml.
5. 200×STP-HRP solution-0.06 ml.
6. Mouse LCN2 standard-10 ng of recombinant mouse LCN2 in a buffered protein base, lyophilised.
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.

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 mouse LCN2 microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

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 (30 ml) with 120 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. The 1×Assay buffer may be stored at 2-8°C for up to one month.

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

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 μ l 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.

D. **1×STP-HRP solution.**

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 μ l of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Mouse LCN2 standards: Reconstitute the lyophilised standard with 1 ml distilled or deionized water. Allow at least 10 minutes for complete reconstitution and invert the vial several times to mix and vortex. This reconstitution produces a stock solution of 10 ng/ml. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
10 ng/ml stock	-	10 ng/ml
250 μ l of 10 ng/ml	250 μ l	5 ng/ml
250 μ l of 5 ng/ml std	250 μ l	2.5 ng/ml
250 μ l of 2.5 ng/ml std	250 μ l	1.25 ng/ml
250 μ l of 1.25 ng/ml	250 μ l	0.625 ng/ml
250 μ l of 0.625 ng/ml	250 μ l	0.312 ng/ml
250 μ l of 0.312 ng/ml	250 μ l	0.156 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml). The reconstituted standard stock should be aliquoted and frozen at -20°C for 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 80-fold dilution in this assay. A suggested dilution step is to add 10 μ l of sample to 790 μ l of 1×Assay buffer. Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1×Assay buffer to prepare these samples.

ASSAY PROCEDURE

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

1. Add 100 μ l 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 μ l of 1 \times Wash buffer to each well and incubate for 1 minute. Discard the 1 \times 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 μ l of 1 \times 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 μ l of 1 \times STP-HRP solution to each well and incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 μ l of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 μ l 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

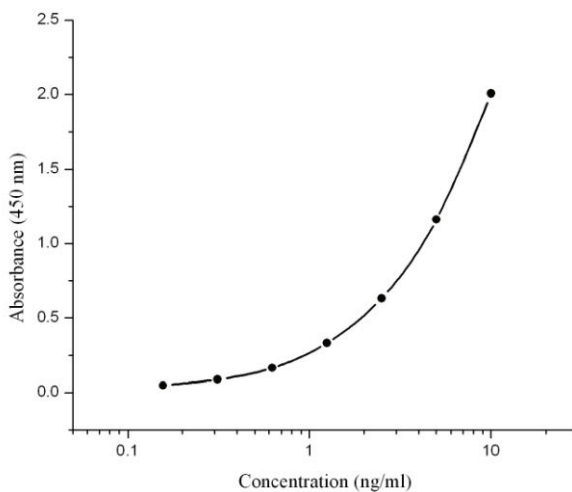
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against mouse LCN2 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine mouse 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.

LCN2 (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.074	0
0.156	0.122	0.048
0.312	0.162	0.088
0.625	0.239	0.165
1.25	0.406	0.332
2.5	0.707	0.633
5	1.236	1.162
10	2.082	2.008

Mouse LCN2 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity: The lowest level of mouse LCN2 that can be detected by this assay is 0.156 ng/ml.

B. Specificity: The antibodies used in this assay are specific to mouse LCN2 and do not cross-react with human LCN2, and other cytokine or hormone molecules.

REFERENCES:

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- [4] Mishra, J. et al. (2005) *Lancet* 365:1231-6.
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- [6] Hanai, J. et al. (2005) *J. Biol. Chem.* 280:13641-7.
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- [8] Wang Y, et al. (2007) *Clin Chem* 53(1):34-41.
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SUMMARY OF ASSAY PROCEDURE

Add 100 μ l of Standard or sample to each well.

☒

Incubate at room temperature for 1 hour.

☒

Aspirate and wash each well three times.

☒

Add 100 μ l of 1 \times Detection antibody solution to each well.

☒

Incubate at room temperature for 1 hour.

☒

Aspirate and wash each well three times.

☒

Add 100 μ l of 1 \times STP-HRP solution to each well.

☒

Incubate at room temperature for 20 minutes.

☒

Aspirate and wash each well four times.

☒

Add 100 μ l of Substrate solution to each well.

☒

Incubate at room temperature for 15 minutes.

☒

Add 100 μ l of Stop solution to each well.

☒

Measure absorbance of each well at 450 nm.

☒

Calculation