Lcn2 (Mouse) ELISA Kit

Catalog Number KA0559

96 assays

Version: 07

Intended for research use only

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Introduction

Intended Use

For quantitative detection of mouse NGAL in cell culture supernates, serum, plasma (heparin) and urine.

Background

Lipocalin-2 (LCN2), also known as NGAL, is a protein associated with neutrophil gelatinase.[1] The LCN2 gene is located at 9q34 and contains 7 exons.[2] The 25-kD LCN2 protein is believed to bind small lipophilic substances such as bacteria-derived lipopolysaccharide (LPS) and formylpeptides and may function as a modulator of inflammation. NGAL tightly binds bacterial catecholate-type ferric siderophores through a cyclically permuted, hybrid electrostatic/cation-pi interaction and is a potent bacteriostatic agent in iron-limiting conditions.[3] The primary LCN2 transcript is 3,696 nucleotides long, and the processed transcript is 809 nucleotides long.[4] LCN2 expression in adult bone marrow, uterus, prostate, salivary gland, stomach, appendix, colon, trachea, and lung, and in fetal spleen and lung. The standard product used in this kit is recombinant mouse NGAL, consisting of 179 amino acids with the molecular mass of 22KDa.

Principle of the Assay

The Lcn2 (Mouse) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from rat specific for NGAL has been precoated onto 96-well plates. Standards (NSO, Q21-N200) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for NGAL is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the mouse NGAL amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
96-well plate precoated with anti-mouse NGAL antibody	96 (8x12) wells
Lyophilized recombinant mouse NGAL standard	10 ng/tube x 2
Biotinylated anti- mouse NGAL antibody, dilution 1:100	130 µL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
- Preparation of 0.01M TBS:
  Add 1.2g Tris, 8.5g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
- Preparation of 0.01 M PBS:
  Add 8.5g sodium chloride, 1.4 g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

Precautions for Use

- ✓ To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.

- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
- ✓ Duplicate well assay is recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ To avoid to use the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Assay Protocol

Reagent Preparation

- ✓ Reconstitution of the mouse NGAL standard: NGAL standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of NGAL standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
- 10,000 pg/mL of mouse NGAL standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- 5000 pg/mL of mouse NGAL standard solution: Add 0.5 mL of the above 10,000 pg/mL NGAL standard solution into 0.5 ml sample diluent buffer and mix thoroughly.
- 2500 pg/mL→78 pg/mL of mouse NGAL standard solutions: Label 6 Eppendorf tubes with 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 313 pg/mL, 156 pg/mL, 78 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 5000 pg/ml NGAL standard solution into 1st tube and mix. Transfer 0.3 mL from 1st tube to 2nd tube and mix. Transfer 0.3 mL from 2nd tube to 3rd tube and mix, and so on.

  Note: The standard solutions are best used within 2 hours. The 10 ng/mL standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- ✓ Preparation of biotinylated anti-mouse NGAL antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- Biotinylated anti-mouse NGAL antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-mouse NGAL antibody to 99 µL antibody diluent buffer.)

- ✓ Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC diluents buffer.)

Sample Preparation

- ✓ Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernate: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.
- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Analyze immediately or aliquot and store samples at -20°C.
- Urine: Aseptically collect the first urine of the day, micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

✓ Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (50-500 ng/mL). The working dilution is 1:100. i.e. Add 3 µL sample into 297 µL sample diluent buffer.
- Medium target protein concentration (5-50 ng/mL). The working dilution is 1:10. i.e. Add 25 µL sample into 225 µL sample diluent buffer.
- Low target protein concentration (78-5000 pg/mL). The working dilution is 1:2. i.e. Add 100 µL sample to 100 µL sample diluent buffer.
- Very Low target protein concentration (≤78 pg/mL). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard NGAL detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of NGAL amount in samples.

1. Aliquot 0.1 mL per well of the 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 313 pg/mL, 156 pg/mL, 78 pg/mL mouse NGAL standard solutions into the precoated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of mouse cell culture supernates, serum, plasma (heparin) or urine to each empty well. See “Sample Dilution Guideline” above for details. It is recommended that each mouse NGAL standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 mL of biotinylated anti-mouse NGAL antibody working solution into each well and incubate the

plate at 37°C for 60 min.

5. Wash the plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 mL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)*
6. Add 0.1 mL of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µL of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min *(Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated mouse NGAL standard solutions; the other wells show no obvious color).*
9. Add 0.1 mL of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

✓ Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse NGAL concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical result

Typical Data Obtained from mouse NGAL

Concentration (pg/mL)	0	78	156	312	625	1250	2500	5,000
O.D	0.070	0.143	0.245	0.494	0.835	1.307	1.969	2.212

(TMB reaction incubate at 37°C for 25 min)

Performance Characteristics

- ✓ Range
  78 pg/mL – 5,000 pg/mL
- ✓ Sensitivity
  < 10 pg/mL
- ✓ Specificity
  Natural and recombinant mouse NGAL
- ✓ Cross-reactivity
  No detectable cross-reactivity with other relevant proteins.

Resources

References

1. Kjeldsen, L.; Johnsen, A. H.; Sengelov, H.; Borregaard, N. : Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J. Biol. Chem. 268: 10425-10432, 1993.
2. Cowland, J. B.; Borregaard, N. : Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 45: 17-23, 1997.
3. Goetz, D. H.; Holmes, M. A.; Borregaard, N.; Bluhm, M. E.; Raymond, K. N.; Strong, R. K. : The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Molec. Cell 10: 1033-1043, 2002.
4. Cowland, J. B.; Borregaard, N. : Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 45: 17-23, 1997.

Plate Layout

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