Human Lipocalin-2/NGAL ELISA Kit

Catalog No. BSKH1048 (96 wells)
For Use with serum, plasma and cell culture supernatants

For Research Use Only. Not for use in diagnostic procedures.

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Introductions

Members of the Lipocalin family have limited sequence identity, but share a highly conserved fold with an eight-stranded anti-parallel β barrel motif that encloses an internal ligand-binding site. They are known for their actions as transporters that carry small hydrophobic molecules such as steroid hormones, vitamins, odorants, and metabolic products. Lipocalin-2, also known as Neutrophil Gelatinase-associated Lipocalin (NGAL) or Siderocalin, was originally identified as a component of neutrophil granules. Since then, its expression has been observed in most tissues, and its synthesis is induced in epithelial cells during inflammation. Lipocalin-2 has been implicated in a variety of cellular processes including the innate immune response, differentiation, tumorigenesis, and cell survival. It is a 25 kDa protein existing in monomeric, homodimeric, and heterodimeric forms, the latter in association with human matrix metalloproteinase 9 (MMP-9). Its association with MMP-9 may modulate protease activity by protecting MMP-9 from degradation. The mouse ortholog (also known as 24p3) shares 62% sequence identity at the amino acid level.

The functions of Lipocalin-2 continue to be elucidated. Studies indicate that it binds bacterial catecholate siderophores bound to ferric ions. This suggests that Lipocalin-2 may act as a bacteriostatic agent by binding bacterial siderophores and limiting bacterial iron supply. This is supported by the observation that mouse Lipocalin-2 is induced in immune cells following Toll-like receptor activation, and Lipocalin-2 mouse knockouts exhibit decreased ability to counter bacterial infection. Lipocalin-2 may also regulate iron uptake into mammalian cells. In the kidney, Lipocalin-2-mediated iron trafficking may be involved in both development and protection from renal injury. Megalin, a member of the LDL receptor family, and 24p3 R have been reported as endocytic receptors for Lipocalin-2. It should be noted that the effects of Lipocalin-2 on cells might be context-dependent. For instance, it has been shown to act as both a survival factor and a pro-apoptotic factor, and its induction by pro-inflammatory cytokines may vary between mouse and human.

Lipocalin-2 has been associated with several pathological processes. For instance, it is upregulated in psoriatic skin in comparison to uninvolved control skin, and Lipocalin-2 suppresses red blood cell production in models of anemia. Lipocalin-2 is elevated in patients with severe acute respiratory syndrome (SARS), and may act as a biomarker for acute renal injury. It has been associated with several tumor types as well, including breast, ovarian, colorectal, and pancreatic cancers. Its function in cancer is unclear, although the invasive and metastatic behavior of tumor cells is suppressed by Lipocalin-2 in models of breast and colon cancer.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Lipocalin-2/NGAL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Lipocalin-2/NGAL present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for Lipocalin-2/NGAL is added to the wells and binds to the combination of capture antibody-Lipocalin-2/NGAL in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of Lipocalin-2/NGAL present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven Lipocalin-2/NGAL standard dilutions and Lipocalin-2/NGAL sample concentration determined.

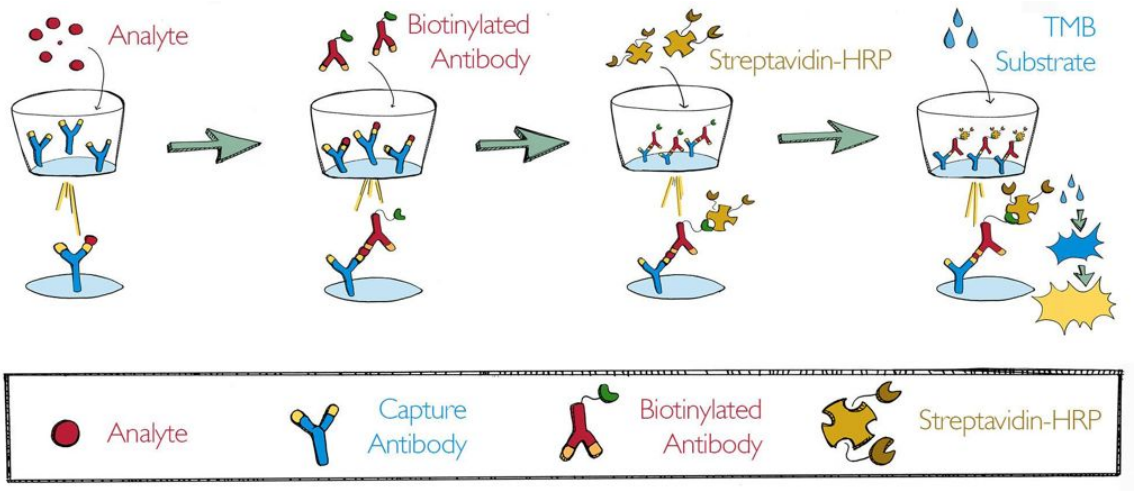


Figure 1. Schematic diagram of the assay

Materials supplied

Table 1. Kit Components

Kit Components	96 wells Quantity/Size
Aluminium pouches with a Microwell Plate coated with monoclonal antibody to human Lipocalin-2/NGAL (8*12)	1 plate
Human Lipocalin-2/NGAL Standard lyophilized, 2500 pg/ml upon reconstitution	2 vials
Concentrated Biotin-Conjugate anti-human Lipocalin-2/NGAL monoclonal antibody	2 vials
Streptavidin-HRP solution	2 vials
Standard /sample Diluent	2 bottles
Biotin-Conjugate antibody Diluent	1 bottle
Streptavidin-HRP Diluent	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 vial
Stop Solution	1 vial
Adhesive Films	4 pieces
Product data sheet	1 copy

Storage

Table 2. Storage of the kit

Unopened Kit	Store at 2 - 8°C. Do **NOT** use past kit expiration date**!**	
Opened/ Reconstituted Reagents	Standard /Sample Diluent	May be stored for up to 1 month at 2 - 8°C.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP Solution	
	Biotin-Conjugate Antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	

	Stop Solution	
	Standard	Aliquot and store for up to 1 month at ≤20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.**

**Provided this is within the expiration date of the kit.

Materials Needed but Not Supplied

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37℃ incubator.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

Precautions for Use

1. Store kit reagents between 2℃and 8℃.
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

Sample Collection and Storage

1. **Cell Culture Supernatants** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor). **Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.**

Reagent Preparation

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 0.5mL of Standard /sample Diluent. This reconstitution produces a stock solution of 5000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500µL of Standard/sample Diluent into the 2500 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

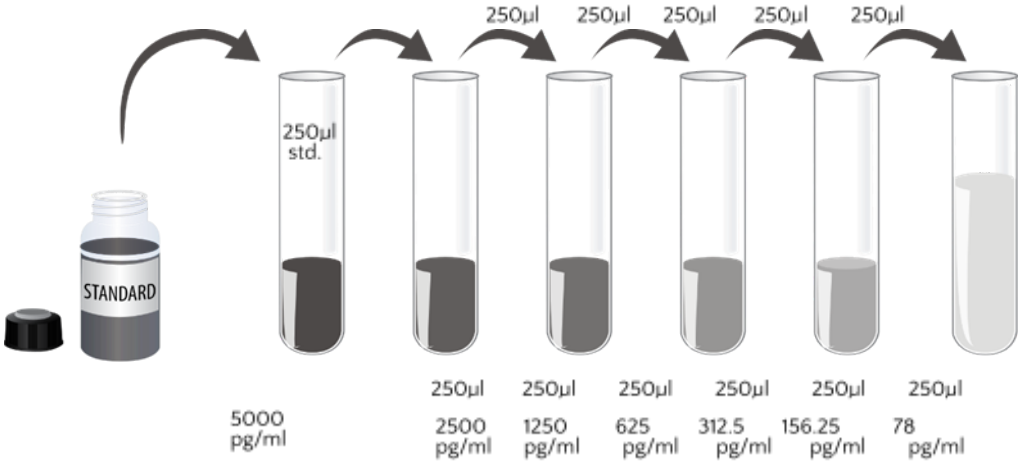


Figure 2. Preparation of Lipocalin-2/NGAL standard dilutions

4. Working solution of Biotin-Conjugate anti-human Lipocalin-2/NGAL monoclonal antibody:

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution

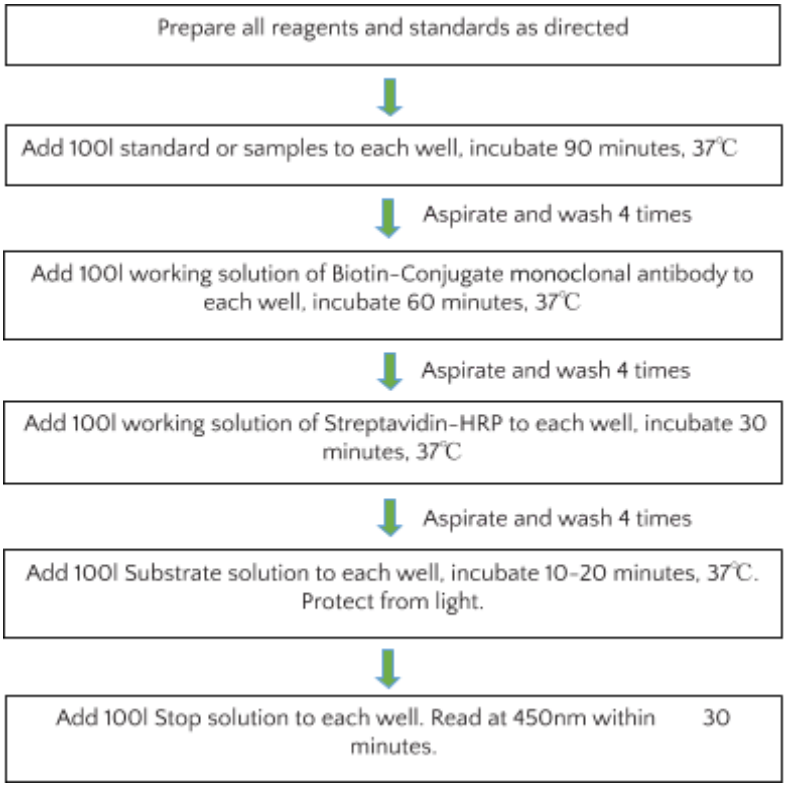
with the Streptavidin-HRP Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

General ELISA Protocol

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100μL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 μL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wavelength; 610-650nm is acceptable)

Assay Procedure Summary



Technical Hints

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the Lipocalin-2/NGAL concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve below is provided ***for demonstration only***. A standard curve should be generated for each set of samples assayed.

Table 3. Typical data using the Lipocalin-2/NGAL ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.022	0.021	0.021	——
78	0.088	0.093	0.090	0.069
156	0.144	0.139	0.141	0.120
312.5	0.261	0.278	0.269	0.248
625	0.452	0.435	0.443	0.422
1250	0.799	0.756	0.777	0.756
2500	1.451	1.423	1.437	1.416
5000	2.323	2.345	2.334	2.313

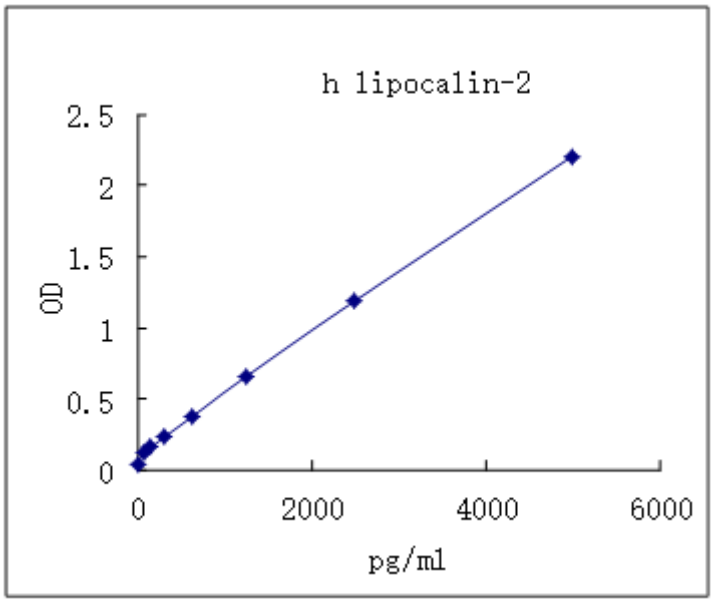


Figure 4. Representative standard curve for human Lipocalin-2/NGAL ELISA.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Performance Characteristics

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY: The minimum detectable dose was 40pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human Lipocalin-2/NGAL. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 4. Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse
COX-2	Lipocalin-2
Lipocalin-1	
MMP-9	