# Human NGAL Immunoassay

**Catalog Number: EA800093**

For the quantitative determination of human NGAL concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

**MANUFACTURED AND DISTRIBUTED BY:**

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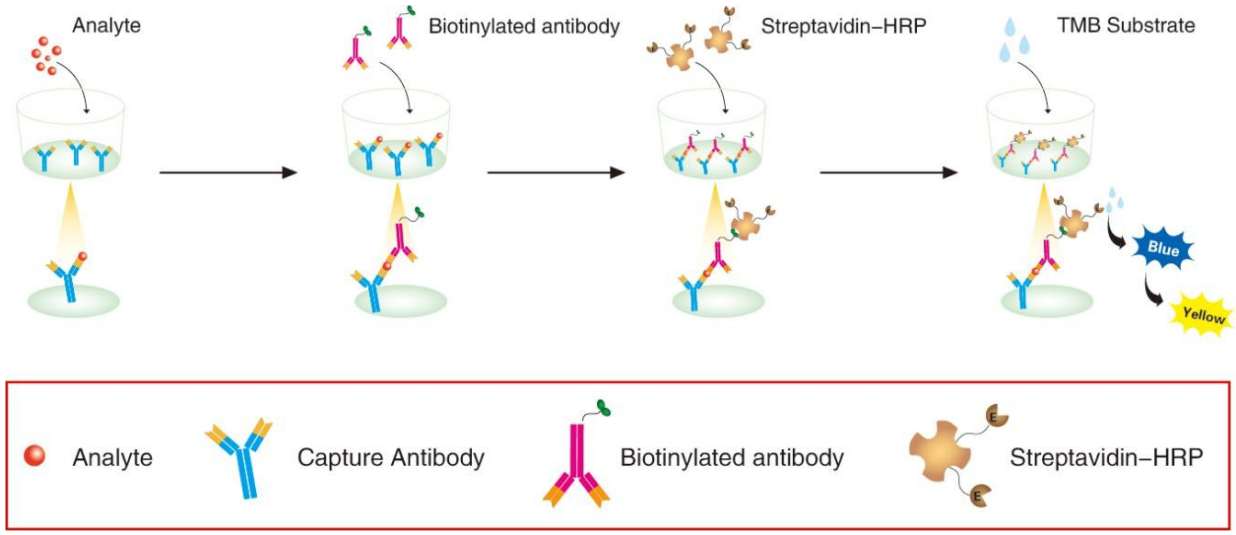
# BACKGROUND

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.

# PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for NGAL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NGAL present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for NGAL is added to detect the captured NGAL protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

## Schematic diagram:




# TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

## PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## KIT COMPONENTS& STORAGE CONDITIONS

| PART | SIZE | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|
| **Microwell Plate** - antibody coated 96-well Microplate (8 wells ×12 strips) | 1 plate | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃** |
| **Standard** - lyophilized,5000 pg/ml upon reconstitution | 2 vials | Aliquot and Store at -20°C** for six months |
| **Concentrated Biotin-Conjugated antibody**(100X) - 120 ul/vial | 1 vial | Store at 2-8°C **for six months |
| **Concentrated Streptavidin-HRP solution**(100X) - 120 ul/vial | 1 vial | Store at 2-8°C** for six months |
| **Standard/Sample Diluent** - 16 ml/ vial | 1 bottle | Store at 2-8°C** for six months |
| **Biotin-Conjugate antibody Diluent** - 16 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Streptavidin-HRP Diluent** - 16 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Wash Buffer Concentrate** (20x) - 30 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Substrate Solution** - 12 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Stop Solution** - 12 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Plate Cover Seals** | 4 pieces | |

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

## OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

## SPECIMEN COLLECTION & STORAGE

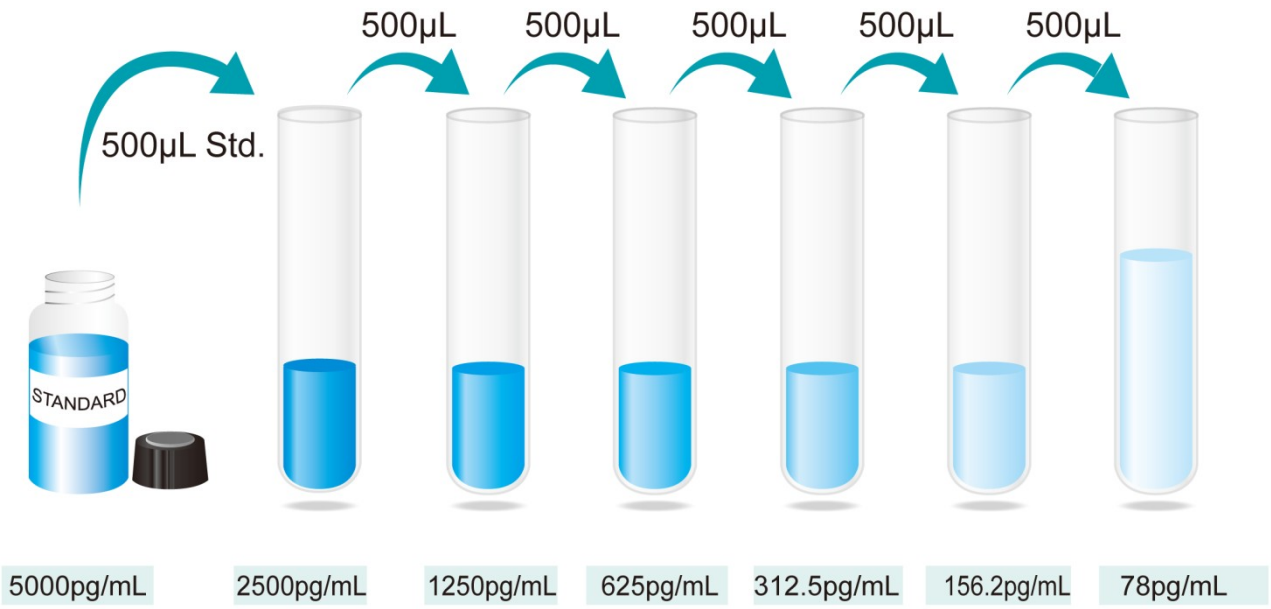
**Cell Culture Supernates** - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
**Serum** - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
**Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.**

## REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL 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/Specimen** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 5000pg/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 2500pg/ml tube and the remaining tubes. Use the stock solution of 5000pg/mL 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



**Preparation of NGAL standard dilutions**

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

4. **Working solution of Biotin-Conjugate anti-human NGAL 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.**

## ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-human NGAL antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 15 minutes,37℃.Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

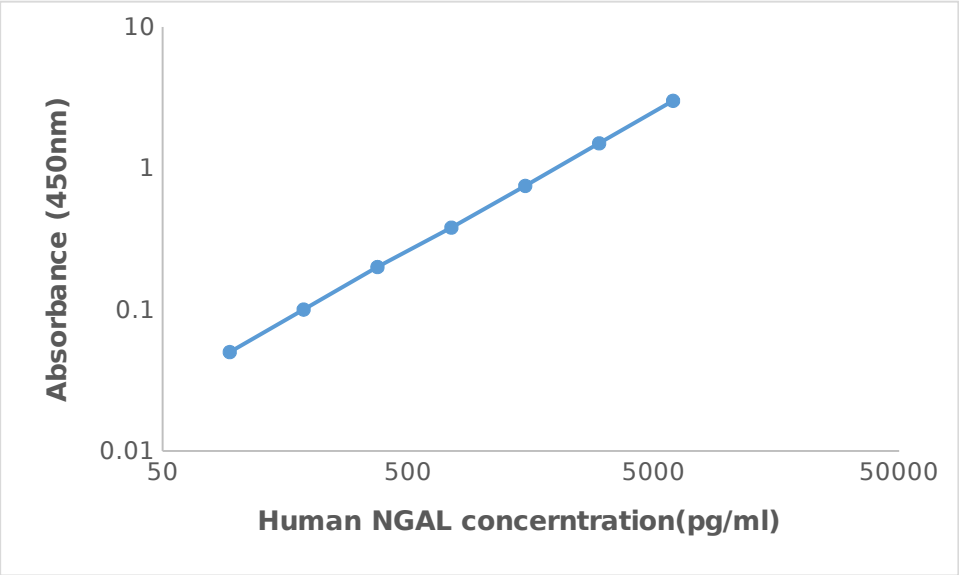
## CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

**Typical data using the NGAL ELISA**

| Standard(pg/ml) | OD. | OD. | Average | Corrected |
|---|---|---|---|---|
| 0 | 0.06 | 0.062 | 0.061 | --- |
| 78.1 | 0.082 | 0.086 | 0.084 | 0.023 |
| 156.2 | 0.153 | 0.162 | 0.157 | 0.096 |

| | | | | |
|---|---|---|---|---|
| 312.5 | 0.268 | 0.296 | 0.282 | 0.221 |
| 625 | 0.503 | 0.506 | 0.504 | 0.443 |
| 1250 | 0.821 | 0.846 | 0.833 | 0.772 |
| 2500 | 1.392 | 1.375 | 1.383 | 1.322 |
| 5000 | 2.354 | 2.385 | 2.369 | 2.308 |




**Representative standard curve for NGAL ELISA.**

## Performance Characteristics

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

**SPECIFICITY:** This assay recognizes both natural and recombinant human NGAL. The factors

listed below were prepared at 100ng/ml in Standard /Sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

**Factors assayed for cross-reactivity**

| Recombinant human | Recombinant mouse | Recombinant porcine |
|---|---|---|
| COX-2 | Lipocalin-2 | |
| Lipocalin-1 | | |
| MMP-9 | | |
| | | |
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**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.

**RECOVERY：** The recovery of NGAL spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

**Recovery of NGAL in two matrices**

| Sample Type | Average % of Expected Range (%) | Range (%) |
|---|---|---|
| Citrate plasma | 90 | 84-102 |
| Cell culture supernatants | 105 | 93-112 |

**LINEARITY:** To assess the linearity of the assay, three samples were spiked with high concentrations of NGAL in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

| Dilution ratio | Recovery (%) | Citrate plasma | Cell culture supernatants |
|---|---|---|---|
| 1:2 | Average% of Expected | 95 | 105 |
| | Range (%) | 82-103 | 95-116 |
| 1:4 | Average% of Expected | 103 | 106 |
| | Range (%) | 95-117 | 102-113 |
| 1:8 | Average% of Expected | 94 | 109 |
| | Range (%) | 82-105 | 99-118 |
| 1:16 | Average% of Expected | 92 | 107 |
| | Range (%) | 83-101 | 97-118 |

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