



## Product Information Sheet

### Rat Lipocalin-2/NGAL ELISA Kit

<b>Catalog No.</b>	EK0855
<b>Size</b>	96T
<b>Range</b>	156pg/ml-10,000pg/ml
<b>Sensitivity</b>	< 15pg/ml

#### Specificity

No detectable cross-reactivity with any other cytokine.

#### Storage

Store at 4°C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

#### Expiration

Four months at 4°C and eight months at -20°C.

#### Application

For quantitative detection of rat NGAL in tissue lysates or cell culture supernates. Sera and plasma have not researched completely.

#### Principle

Rat NGAL ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat NGAL specific-specific polyclonal antibodies were precoated onto 96-well plates. The rat specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells 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 rat NGAL amount of sample captured in plate.

#### Kit Components

1. Lyophilized recombinant rat NGAL standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- rat NGAL antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- rat NGAL antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

#### Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6.

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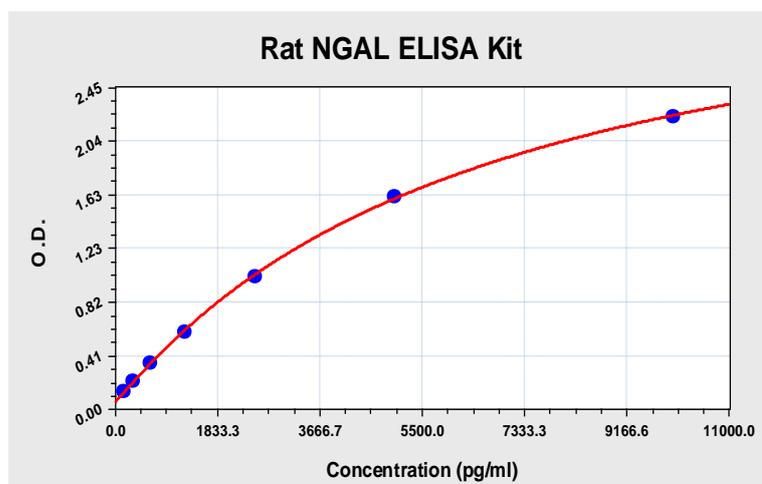
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Finally, adjust the total volume to 1L.

## Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. 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.

## Rat NGAL ELISA Kit-1X96 Well Plate Image



## Background

Lipocalin-2 (LCN2), also known as NGAL, is a protein associated with neutrophil gelatinase.<sup>1</sup> The LCN2 gene is located at 9q34 and contains 7 exons.<sup>2</sup> 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.<sup>3</sup> The primary LCN2 transcript is 3,696 nucleotides long, and the processed transcript is 809 nucleotides long.<sup>4</sup> 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 rat NGAL, consisting of 177 amino acids with the molecular mass of 21kDa.

## Reference

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.

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