

**DESCRIPTION**

**Source** Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey Lipocalin-2/NGAL protein  
Gln21-Gly198  
Accession # XP\_005580845.2  
with C-terminal 6-His tag

**N-terminal Sequence Analysis** No results obtained. Gln21 is inferred from enzymatic pyroglutamate treatment revealing Asp22.

**Predicted Molecular Mass** 21 kDa

**SPECIFICATIONS**

**SDS-PAGE** 21-27 kDa, reducing conditions

**Activity** Measured by its ability to bind Iron(III) dihydroxybenzoic acid [Fe(DHBA)<sub>3</sub>]. The binding of Fe(DHBA)<sub>3</sub> results in the quenching of Trp fluorescence in Lipocalin-2.  
>1.0 μM of Fe(DHBA)<sub>3</sub> can be bound under the described conditions.

**Endotoxin Level** <0.10 EU per 1 μg of the protein by the LAL method.

**Purity** >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Supplied as a 0.2 μm filtered solution in MES and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer: 50 mM Tris, 10 mM CaCl<sub>2</sub>, 150 mM NaCl, pH 7.5 (TCN)
  - Ligand Buffer: 0.1 M Tris, pH 8.0
  - Recombinant Cynomolgus Monkey Lipocalin-2/NGAL His-tag (cynoLipocalin-2) (Catalog # 2357-LC)
  - Iron III (Fe<sup>3+</sup>) (Sigma, Catalog # 16596)
  - 2,3-Dihydroxybenzoic Acid (DHBA) (Sigma, Catalog # 126209)
  - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
  - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Prepare a curve of Fe<sup>3+</sup> in deionized water with the following serial dilutions: 640, 320, 160, 80, 40, 20, 10, 5, and 2.5 μM.
  2. Prepare 1 mM DHBA in Ligand Buffer from powder stock.
  3. Combine equal volumes of the Fe<sup>3+</sup> curve with 1 mM DHBA. Include a control containing 1 mM DHBA and Ligand Buffer.
  4. Incubate at room temperature for 10 minutes. A curve of the metal ligand complex of Fe(DHBA)<sub>3</sub> is formed.
  5. After incubation, perform 5 fold dilutions to the curve using Assay Buffer.
  6. Dilute rcynoLipocalin-2 (MW: 21200 Da) to 4 μM in Assay Buffer.
  7. In the plate, load 50 μL of the diluted Fe(DHBA)<sub>3</sub> complex curve and 50 μL of 4 μM rcynoLipocalin-2.
  8. Incubate at room temperature for 30 minutes.
  9. Read at excitation and emission wavelengths of 280 nm and 340 nm, respectively in endpoint mode.
  10. Plot a 4-parameter curve of Fe(DHBA)<sub>3</sub> Concentration (x-axis) versus RFUs (y-axis), and calculate a BC50 from the curve.

- Final Assay Conditions**
- Per Well:
- Fe(DHBA)<sub>3</sub> Complex Curve: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, and 32 μM.
  - rcynoLipocalin-2: 2 μM

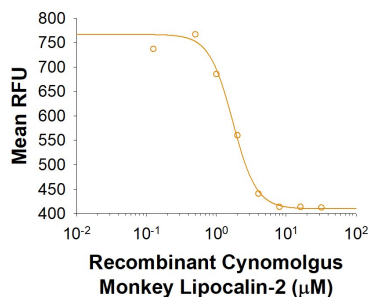
**PREPARATION AND STORAGE**

**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

- Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
  - 3 months, -20 to -70 °C under sterile conditions after opening.

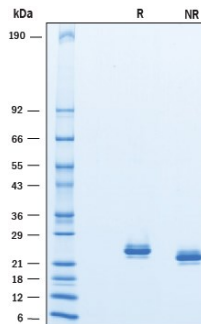
**DATA**

**Binding Activity**



Recombinant Cynomolgus Monkey Lipocalin-2 (Catalog # 2357-LC) is measured by its ability to bind Iron(III) dihydroxybenzoic acid.

**SDS-PAGE**



2 µg/lane of Recombinant Cynomolgus Monkey Lipocalin-2/NGAL was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a primary band at 24 kDa under reducing conditions.

**BACKGROUND**

Lipocalin-2 (LCN-2) is a member of the lipocalin family. These proteins share a highly conserved fold with an eight-stranded antiparallel  $\beta$  barrel and act as small molecule transporters (1). Lipocalin-2, also known as Neutrophil Gelatinase-Associated Lipocalin (NGAL), was originally identified as a component of neutrophil granules (2). It is a 25 kDa protein existing in monomeric and homo- and heterodimeric forms, the latter as a dimer with neutrophil gelatinases (MMP-9) (2). Studies indicate that Lipocalin-2 binds iron through association with dihydroxybenzoic acid (DHBA), a siderophore similar to bacterial enterobactin (3). Its expression has been observed in most tissues normally exposed to microorganisms and is induced in epithelial cells during inflammation (2). Secretion of Lipocalin-2 in immune cells increases by stimulation of Toll-like receptor as an acute phase response to infection. As a result, it acts as a potent bacteriostatic reagent by sequestering iron (4). Lipocalin-2 has been implicated in a variety of processes including cell differentiation, tumorigenesis, and apoptosis (5-8). Lipocalin-2 can alter the invasive and metastatic behavior of Ras-transformed breast cancer cells via effects on the Ras-MAPK signaling pathway (9). In the kidney, Lipocalin-2 mediated iron trafficking may be involved in renal injury, and it has been implicated as a marker for early kidney failure (10-12).

**References:**

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