Recombinant Cynomolgus Monkey Lipocalin-2/NGAL His-tag

Catalog Number: 2357-LC

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 [$Fe(DHBA)_3$]. The binding of $Fe(DHBA)_3$ results in the quenching of Trp fluorescence in Lipocalin-2. >1.0 µM of $Fe(DHBA)_3$ 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 $CaCl_2$, 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^{3+}) (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^{3+} 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^{3+} curve with 1 mM DHBA. Include a control containing 1 mM DHBA and deionized water.
4. Incubate at room temperature for 10 minutes. A curve of the metal ligand complex of $Fe(DHBA)_3$ 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 $Fe(DHBA)_3$ 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 $Fe(DHBA)_3$ Concentration (x-axis) versus RFUs (y-axis), and calculate a BC50 from the curve.

Final Assay Conditions

Per Well:

- $Fe(DHBA)_3$ 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

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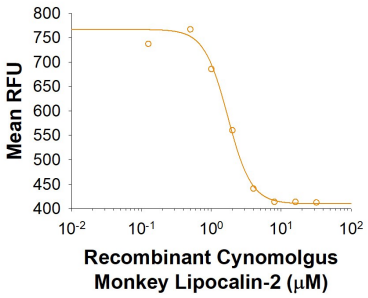
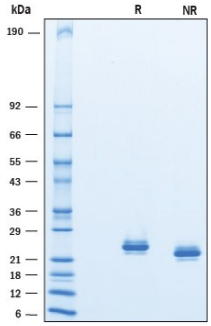
Binding Activity



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 β 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 sidropore 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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