Recombinant Mouse Lipocalin-2/NGAL

Catalog Number: 1857-LC

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Gln21-Asn200 with a C-terminal 10 His tag Accession # P11672
N-terminal Sequence Analysis	No results obtained: Gln21 predicted
Predicted Molecular Mass	22 kDa

SPECIFICATIONS

SDS-PAGE	26-27 kDa doublet, 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	<1.0 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 Mouse Lipocalin-2/NGAL (rmLipocalin-2) (Catalog # 1857-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 of 1 mM DHBA and Ligand Buffer.
4. Incubate at room temperature for 10 minutes.
5. After incubation, perform 5 fold dilutions to the curve with Assay Buffer.
6. Dilute rmLipocalin-2 (MW: 21905 Da) to 4 µM in Assay Buffer.
7. In a plate, load 50 µL of the diluted $Fe(DHBA)_3$ complex curve and 50 µL of 4 µM rmLipocalin-2.
8. Incubate reaction 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 BC_{50} from the curve.

Final Assay Conditions

Per Well:

- $Fe(DHBA)_3$ complex: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, and 32 µM.
- rmLipocalin-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.

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BACKGROUND

Mouse Lipocalin-2 was cloned from mouse kidney cells (1). Its very high level of expression at the post-stratum uterus gave it the name uterocalin (2). Lipocalin-2 has been implicated in a variety of processes including cell differentiation, tumorigenesis, and apoptosis (3-5). Studies indicate that Lipocalin-2 binds a bacterial catecholate siderophore that is bound to a ferric ion, such as enterobactin, with a subnanomolar dissociation constant (K_D = 0.41 nM) (6). The bound ferric enterobactin complex breaks down slowly in a month into dihydroxybenzoyl serine and dihydroxybenzoic acid (DHBA). It also binds to a ferric DHBA complex with much less K_D values (7.9 nM) (6). Secretion of Lipocalin-2 in immune cells increases in response to 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 (7). Moreover, Lipocalin-2 can alter the invasive and metastatic behavior of Ras-transformed breast cancer cells *in vitro* and *in vivo* by reversing the epithelial to mesenchymal transition inducing activity of Ras, through restoration of E-cadherin expression, via effects on the Ras-MAPK signaling pathway (8).

References:

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