**DESCRIPTION**

**Source**
Mouse myeloma cell line, NS0-derived human Lipocalin-2/NGAL protein
Gln21-Gly198, with a C-terminal 10-His tag
Accession #: P80188

**N-terminal Sequence Analysis**
No results obtained; Gln21 predicted

**Predicted Molecular Mass**
22 kDa

**SPECIFICATIONS**

**SDS-PAGE**
25-27 kDa doublet, reducing conditions

**Activity**
Measured by its ability to bind Iron(III) dihydroxybenzoic acid \([\text{Fe(DHBA)}_3]\). The binding of \([\text{Fe(DHBA)}_3]\) results in the quenching of Trp fluorescence in Lipocalin-2.
rhLipocalin-2 can bind >1.5 µM of \([\text{Fe(DHBA)}_3]\) under the described conditions.

**Endotoxin Level**
<1.0 EU per 1 µg of the protein by the LAL method.

**Purity**
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**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)
- Recombinant Human Lipocalin-2/NGAL (rhLipocalin-2) (Catalog #: 1757-LC)
- Iron III \((\text{Fe}^{3+})\) (Sigma, Catalog #: 16596)
- 2,3 Dihydroxybenzoic Acid (DHBA) (Sigma, Catalog #: 126209)
- Ligand Buffer: 0.1 M Tris, pH 8.0
- 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 \(\text{Fe}^{3+}\) 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 \(\text{Fe}^{3+}\) 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 \([\text{Fe(DHBA)}_3]\) is formed.
5. After incubation, perform 5 fold dilutions to the curve using Assay Buffer.
7. In the plate, load 50 µL of the diluted \([\text{Fe(DHBA)}_3]\) complex curve and 50 µL of 4 µM rhLipocalin-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 \([\text{Fe(DHBA)}_3]\) concentration (x-axis) versus RFUs (y-axis), and calculate a BC\(_{50}\) from the curve.

**Final Assay Conditions**

**Per Well:**
- \([\text{Fe(DHBA)}_3]\) Curve: 0.001, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, and 32 µM.
- rhLipocalin-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.
Members of Lipocalin family share a highly conserved fold with an eight-stranded antiparallel β barrel, and act as transporters, carrying small molecules to specific cells (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 human neutrophil gelatinases (MMP-9) (2). Its expression has been observed in most tissues normally exposed to microorganism, and its synthesis is induced in epithelial cells during inflammation (3). 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 sidropore bound to ferric ion such as enterobactin with a subnanomolar dissociation constant \( K_d = 0.41 \text{ nM} \) (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 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 (7). Moreover, Lipocalin-2 can alter the invasive and metastatic behavior of Ras-transformed breast cancer cells in vitro and in vivo by reversing epithelial to mesenchymal transition inducing activity of Ras, through restoration of E-cadherin expression, via effects on the Ras-MAPK signaling pathway (8).

References: