Recombinant Human Hemopexin
Catalog Number: 4490-HP

DESCRIPTION

Source
Mouse myeloma cell line, NS0-derived
Thr24-His462, with a C-terminal 6-His tag
Accession # NP_000604

N-terminal Sequence
Analysis
Thr24

Predicted Molecular Mass
50 kDa

SPECIFICATIONS

SDS-PAGE
71 kDa, reducing conditions

Activity
Measured by its ability to bind protoporphyrin IX (PPP-IX).
Recombinant Human Hemopexin binds >6 μM PPP-IX, resulting in a 50% decrease in the fluorescence signal of rhHemopexin as measured under the described conditions.

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

Purity
>90%, 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 A: 0.1 M Sodium Borate, pH 9.0
- Assay Buffer B: 5 mM NaOH in 50% (v/v) Glycerol and 50% (v/v) Methanol solution
- Assay Buffer C: 50% Assay Buffer A, 50% Assay Buffer B
- Recombinant Human Hemopexin (rhHemopexin) (Catalog # 4490-HP)
- Protoporphyrin IX (PPP-IX) (Sigma, Catalog # P8293) Prepare 10 mM Stock in DMSO
- F16 Black Maxiisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhHemopexin to 60 μg/mL in Assay Buffer A.
2. Dilute PPP-IX to 640 μM in Assay Buffer B.
3. Dilute the 640 μM PPP-IX to 320 μM in Assay Buffer A.
4. Prepare a curve of PPP-IX in Assay Buffer C. Make the following serial dilutions starting with the dilution made in the previous step: 320, 160, 80, 40, 20, 10, 5, 2.5, and 1.25 μM.
5. Mix equal volumes of the PPP-IX curve dilutions and the 60 μg/mL rhHemopexin. Include a control containing Assay Buffer C and the 60 μg/mL rhHemopexin.
6. Incubate mixtures at room temperature for 30 minutes.
7. Load 100 μL of reaction mixtures and control into a plate.
8. Read at excitation and emission wavelengths of 280 nm and 340 nm (top read), respectively in endpoint mode.
9. Plot the curve to obtain the concentration of PPP-IX that results in 50% decrease in the fluorescence signal from the control.

Final Assay Conditions
Per Well:
- rhHemopexin: 30 μg/mL (0.6 μM)
- PPP-IX curve: 160, 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 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.

BACKGROUND

Hemopexin (HPX) is a 60 kDa plasma glycoprotein with two four-bladed β-propeller folds. This structural motif has been found in other proteins including collagenases and provides sites for protein-protein interactions (1-3). The liver is the major synthesizing organ. Expression in the central nervous system, in the retina, and in peripheral nerves has also been observed. Hemopexin belongs to the family of the acute-phase proteins whose synthesis is induced after an inflammatory event. Hemopexin participates in maintaining and recycling the iron pool by utilizing its high binding affinity toward heme composed of protoporphyrin IX and iron. It also functions in preventing oxidation caused by heme after hemolysis. Hydrophobic heme molecules can intercalate into lipid membranes and participate in the oxidation of lipid membrane components through the Fenton reaction resulting in lipid peroxidation. Hemopexin undergoes a conformational change upon the binding of heme. The conformational change allows hemopexin to interact with a specific receptor, forming a complex which is then internalized. In the plasma, it is likely that heme binds albumin (35-55 g/L) first because of the higher concentration of albumin in plasma than hemopexin (0.5-1.2 g/L), and is then transferred to hemopexin, which has a much higher affinity (Kd = 1 pM) toward heme. Heme concentrations in plasma increase after hemolysis, which is associated with several pathological conditions such as reperfusion injury and ischemia.

References: