R&D SYSTEMS
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Recombinant Human HO-1/HMOX1/HSP32

Catalog Number: 3776-HM

DESCRIPTION

Source	*E. coli*-derived Met1-Thr261 (Phe33Leu), with a C-terminal 6-His tag Accession # P09601
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	31 kDa

SPECIFICATIONS

SDS-PAGE	29-31 kDa, reducing conditions
Activity	Measured by its ability to oxidize hemin to biliverdin. The specific activity is >5 pmol/min/µg, as measured 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 Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Human HO-1/HMOX1/HSP32 (rhHO-1) (Catalog # 3776-HM)
- Recombinant Human POR/Cytochrome P450 Reductase (rhPOR) (Catalog # 6340-PR)
- Recombinant Human Biliverdin Reductase A/BLVRA (rhBLVRA) (Catalog # 6454-BR)
- Catalase (Sigma, Catalog # C30)
- Hemin (Sigma, Catalog # H9039), 10 mM stock in DMSO
- Bovine Serum Albumin (BSA), 100 mg/mL stock in deionized water
- β-Nicotinamide adenine 2'-phosphate reduced tetrasodium salt hydrate (NADPH) (Sigma, Catalog # N7505), 10 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute Hemin to 1 mM in Assay Buffer.
2. Prepare Reaction Mixture containing 60 µM Hemin, 4 mg/mL BSA, 1000 U/mL catalase, 80 µg/mL rhPOR, and 80 µg/mL rhBLVRA in Assay Buffer.
3. Prepare 80 µg/mL rhHO-1 in Assay Buffer.
4. Dilute NADPH to 1 mM in Assay Buffer.
5. In a clear plate, load 25 µL dilute rhHO-1 and add 25 µL Reaction Mixture. Include an enzyme blank containing 25 µL Assay Buffer and 25 µL Reaction Mixture.
6. Start the reaction by adding 50 µL 1 mM NADPH to all wells used.
7. Read absorbance at 468 nm (bottom read) in kinetic mode for 5 minutes.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 43500 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Reaction:

- rhHO-1: 2 µg
- rhPOR: 2 µg
- rhBLVRA: 2 µg
- Catalase: 250 U/mL
- Hemin: 15 µM
- BSA: 1 mg/mL
- NADPH: 0.5 mM

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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

Heme oxygenase (HMOX) is the rate limiting enzyme in heme catabolism (1). It cleaves heme to biliverdin, carbon monoxide, and iron. The biliverdin is subsequently converted to bilirubin by biliverdin reductase. The mechanism of HMOX is unique in that heme serves as the substrate of the enzyme and as the prosthetic group for the activation of iron-bound O_2. HMOX activity is highest in spleen where senescent erythrocytes are sequestered and destroyed. Two isoforms, HMOX1 and HMOX2, are expressed in most tissues. HMOX1 is an inducible enzyme in response to heme, heavy metals, oxidative stress, cytokines, and many drugs (2). Whereas HMOX2 displays a constitutive expression. HMOX1 is expressed mainly in spleen, liver, and kidney, and HMOX2 is prominently expressed in the brain and testes. The increased expression of HMOX1 levels is related to a variety of pathological states, where it functions as a cytoprotective molecule through its by-products (3). HMOX1 also plays important roles in the regulation of cell proliferation, differentiation, and apoptosis.

References:

1. Kappas, A. (2008) Pharmacol. Rev. **60**:79.
2. Otterbein, L.E. and Choi, A.M.K. (2000) Am. J. Physiol. Lung Cell. Mol. Physiol. **279**:1029.
3. Grochot-Przeczek, A. *et al*. (2012) Clin. Sci. (Lond) **122**:93.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
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