Recombinant Human QSOX1/Quiescin Q6

Catalog Number: 9209-QS

DESCRIPTION

Source	*E. coli*-derived Ser33-Ala546, with an N-terminal Met and 6-His tag Accession # O00391
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	58 kDa

SPECIFICATIONS

SDS-PAGE	55 kDa, reducing conditions
Activity	Measured by its ability to produce hydrogen peroxide during the oxidation of Dithiothreitol (DTT). The specific activity is >1,200 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Phosphate, pH 7.5
- Recombinant Human QSOX1/Quiescin Q6 (rhQSOX1) (Catalog # 9209-QS)
- Coupling Enzyme: Horseradish Peroxidase (HRP) (250-330 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: Dithiothreitol (DTT) (Amresco, Catalog # 0281), 1 M stock in deionized water
- Substrate Component 2: Amplex® Ultra Red (AUR) (Invitrogen, Catalog # A36006), 10 mM stock in DMSO
- F15 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhQSOX1 to 1 ng/µL in Assay Buffer.
2. Prepare a Substrate Mixture containing 100 µM AUR, 2 units/mL HRP, and 300 µM DTT in Assay Buffer. Make sure to add DTT to the Substrate Mixture right before loading the plate.
3. Load 50 µL of 1 ng/µL rhQSOX1 into the plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read at excitation and emission wavelengths of 544 nm and 590 nm in kinetic mode for 5 minutes. Note: A cutoff must be set at a wavelength of 570 nm.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using a fluorescent standard prepared by incubating 50 µM AUR, 1 unit/mL HRP and 150 µM DTT and a curve of Hydrogen Peroxide (Sigma, Catalog # H1009) in Assay Buffer. Use this oxidized AUR curve to determine the conversion factor.

Final Assay Conditions

Per Well:

- rhQSOX1: 0.05 µg
- DTT: 150 µM
- HRP: 1 unit/mL
- AUR: 50 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

Sulfhydryl Oxidase-1 (QSOX1) is an approximately 80 kDa enzyme that contains thioredoxin and sulfhydryl oxidase domains (1-3). It is synthesized with a C-terminal transmembrane segment, but soluble secreted forms can be generated by alternative splicing or proteolytic shedding within the Golgi (4). Within the region encompassing both enzymatic domains and the central region, human QSOX1 shares 79% aa sequence identity with mouse and rat QSOX1. It plays a role nascent protein folding by mediating disulfide oxidation (4-6). This activity is required for Laminin incorporation into the extracellular matrix (7). QSOX1 is up-regulated in many cancers and supports tumor cell proliferation and invasion (1, 8).

References:

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