Recombinant Human POR/Cytochrome P450 Reductase His-tag

Catalog Number: 6340-PRB

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human POR/Cytochrome P450 Reductase protein Ser62-Ser677 with an N-terminal Met and 6-His tag Accession # P16435.2
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	71 kDa

SPECIFICATIONS

SDS-PAGE	63-69 kDa, under reducing conditions.
Activity	Measured by the reduction of cytochrome c using NADPH as the cofactor. The specific activity is >1800 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.3 M Potassium Phosphate, pH 8.0
- Recombinant Human POR/Cytochrome P450 Reductase His-tag (rhPOR) (Catalog # 6340-PRB)
- Substrate: β-NADPH, 10 mM stock in deionized water
- Cytochrome C, Bovine heart, 2 mg/mL stock in deionized water
- Clear 96 well Plate (Catalog # DY990)
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute rhPOR to 0.2 µg/mL in Assay Buffer.
2. Prepare a Substrate Mixture containing 200 µM β-NADPH and 1 mg/mL Cytochrome C in Assay Buffer.
3. Load 50 µL of 0.2 µg/mL rhPOR, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate Mixture.
4. Read in kinetic mode for 6 minutes with 1 minute lag time at an absorbance of 550 nm.
5. 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 21100 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhPOR: 0.010 µg
- Cytochrome C: 0.5 mg/ml
- β-NADPH: 100 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.

DATA

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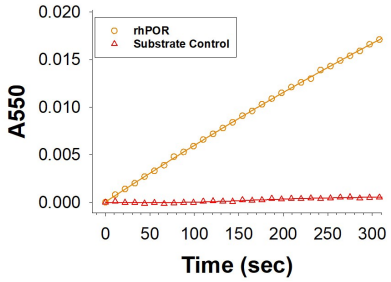
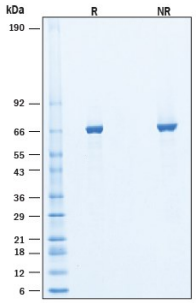
Enzyme Activity



Recombinant Human POR/Cytochrome P450 Reductase His-tag Protein Enzyme Activity. Recombinant Human POR His-tag (Catalog # 6340-PRB) is measured by the reduction of cytochrome c using NADPH as the cofactor.

SDS-PAGE



Recombinant Human POR/Cytochrome P450 Reductase His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human POR/Cytochrome P450 Reductase His-tag Protein (Catalog # 6340-PRB) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 63-69 kDa, under reducing conditions.

BACKGROUND

NADPH-Cytochrome P450 Reductase (P450R), or POR, is an essential component of the cytochrome P450 monooxygenase system of eukaryotic cells (1). P450R is anchored in the endoplasmic reticulum membrane with its catalytic domain residing in the cytosol. The protein is composed of four domains: an N-terminal FMN-binding domain, a connecting domain, and C-terminal FAD- and NADPH-binding domains (2). The flavoprotein P450R contains one molecule each of FMN and FAD, which are essential for the transfer of electrons from NADPH to the P450 cytochromes (3). This reduction is necessary for cytochromes P450 to perform each cycle of oxidation. P450R plays a vital role in metabolism and detoxification of xenobiotic and endobiotic compounds (4). P450R is also capable of transferring electrons to cytochrome b5, heme oxygenase, the fatty acid elongation system, and other proteins (4). Mutations of P450R can result in disordered steroidogenesis and bone defects in Antley-Bixler syndrome (5, 6) and expression of P450R has been correlated with patient outcome in breast cancer (7).

References:

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