Recombinant Human POR/Cytochrome P450 Reductase

Catalog Number: 6340-PR

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human POR/Cytochrome P450 Reductase protein Arg45-Ser677, with N-terminal amino acids WAGALA and 6-His tag Accession # P16435.2
N-terminal Sequence Analysis	N-terminal Trp & P16435.2 Ser62
Predicted Molecular Mass	73 kDa & 70 kDa

SPECIFICATIONS

SDS-PAGE	65-80 kDa, reducing conditions
Activity	Measured by the reduction of cytochrome c using NADPH as the cofactor. The specific activity is >6,500 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	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.3 M KH_2PO_4, pH 8.0
- Recombinant Human POR/Cytochrome P450 Reductase (rhPOR) (Catalog # 6340-PR)
- Substrate: β-NADPH (Sigma, Catalog # N7505), 10 mM stock in deionized water (8.33 mg/mL)
- Cytochrome C, Bovine heart (Sigma, Catalog # C3131), 2 mg/mL stock in deionized water
- 96 well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhPOR to 0.2 ng/µL in Assay Buffer.
2. Combine 480 µL of Assay Buffer, 500 µL of 2 mg/mL Cytochrome C, and 20 µL of 10 mM β-NADPH.
3. Load 50 µL of 0.2 ng/µL 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:

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

*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: 0.1 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

NADPH-Cytochrome P450 Reductase (P450R) 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. P450R is a flavoprotein, containing one molecule each of FMN and FAD, which are essential for the transfer of electrons from NADPH to the cytochromes P450 (2). This reduction is necessary for cytochromes P450 to perform each cycle of oxidation. P450R is also capable of transferring electrons to cytochrome b_5, heme oxygenase, the fatty acid elongation system, and other proteins. Mutations of P450R can result in disordered steroidogenesis and Antley-Bixler syndrome.

References:

1. Philips, A.H. and R.G. Langdon (1962) J. Biol. Chem. **237**:2652.
2. Iyanagi, T. and H.S. Mason (1973) Biochemistry **12**:2291.
3. Flueck C.E. *et al.* (2004) Nat. Genet. **36**:228.

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PRODUCT SPECIFIC NOTICES

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