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Product datasheet

Cytochrome P450 Reductase Activity Assay Kit (Colorimetric) ab204704

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Overview

Product name Cytochrome P450 Reductase Activity Assay Kit (Colorimetric)

Detection methodColorimetric

Sample type Cell Lysate, Microsomes, Purified protein, Tissue Lysate

Assay type Enzyme activity
Sensitivity 0.2 mU/well

Species reactivity Reacts with: Mammals, Other species

Product overview Cytochrome P450 Reductase Activity Assay Kit (Colorimetric)

Cytochrome P450 Reductase Activity Assay Kit (Colorimetric) (ab204704) couples the oxidation of NADPH by cytochrome P450 reductase (CPR) to the reduction of a nearly colorless probe into a brightly colored product with an absorbance peak at OD=460 nm, with the rate of color generation being directly proportional to CPR activity. The NADPH utilized by CPR is generated in situ from β -NADP+ via oxidation of glucose-6-phosphate (G6P) to 6-phospho-D-glucono-1,5-lactone by glucose-6-phosphatase dehydrogenase (G6PDH). The kit can be used to determine CPR activity in a variety of samples, with a detection limit of ~0.2 mU of CPR activity per reaction.

For assessment of CPR activity in crude biological samples that may have extraneous reductases capable of reducing the substrate, an inhibitor of NADPH-dependent flavoproteins is included. In this case, the specific CPR activity may be calculated by running parallel reactions in the presence and absence of inhibitor and subtracting any residual activity detected with the inhibitor present. The kit contains sufficient reagents for performing 100 reactions in a 96-well plate format.

This product is manufactured by BioVision, an Abcam company and was previously called K700 Cytochrome P450 Reductase (CPR) Activity Kit (Colorimetric). K700-100 is the same size as the 100 test size of ab204704.

NADPH-cytochrome P450 reductase (CPR, EC 1.6.2.4) is a ~78 kDa membrane-bound flavoenzyme that catalyzes the transfer of electrons from NADPH to members of the cytochrome P450 monooxidase (CYP) enzyme family in the endoplasmic reticulum. CPR contains two tightly bound flavin cofactors, FAD and FMN, which participate in the sequential transfer of electrons from NADPH→FAD→FMN→CYP, oxidizing NADPH to NADP+ and reducing the CYP heme moiety to the substrate- and oxygen-binding ferrous state. As CPR is required for the function of all CYP isozymes, it plays a critical role in the metabolism of drugs, organic pollutants and other xenobiotic compounds, in addition to its role in biosynthesis of certain vitamins and steroid hormones.

Notes

Properties

Storage instructions

Store at -20°C. Please refer to protocols.

Components	100 tests
CPR Assay Buffer	1 x 50ml
CPR Inhibitor	1 x 100µl
CPR Standard (G6P)	1 vial
G6P Standard Developer	1 vial
Human CPR Positive Control	1 vial
NADPH Substrate Mix	1 vial

Function

This enzyme is required for electron transfer from NADP to cytochrome P450 in microsomes. It can also provide electron transfer to heme oxygenase and cytochrome B5.

Involvement in disease

Defects in POR are the cause of adrenal hyperplasia variant type (AHV) [MIM:201750]; also known as Antley-Bixler syndrome-like phenotype with disordered steroidogenesis. AHV is a rare variant of congenital adrenal hyperplasia. It is an autosomal recessive disorder with apparent combined P450C17 and P450C21 deficiency. Affected girls are born with ambiguous genitalia, but their circulating androgens are low and virilization does not progress. Conversely, affected boys are sometimes born undermasculinized. Boys and girls can also present with bone malformations, in some cases resembling the pattern seen in patients with Antley-Bixler syndrome.

Defects in POR are a cause of isolated disordered steroidogenesis (IDS) [MIM:201750].

Sequence similarities

In the C-terminal section; belongs to the flavoprotein pyridine nucleotide cytochrome reductase

family.

Contains 1 FAD-binding FR-type domain.

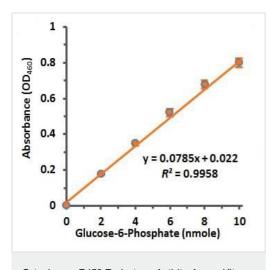
Contains 1 flavodoxin-like domain.

Cellular localization

Endoplasmic reticulum membrane. Anchored to the ER membrane by its N-terminal hydrophobic

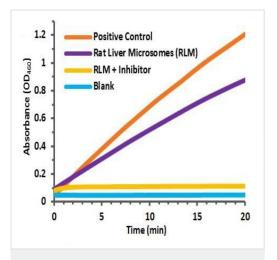
region.

Images



Typical G6P Standard curve using Cytochrome P450 Reductase Activity Assay Kit (Colorimetric) (ab204704). One mole G6P corresponds to one mole of β -NADP+ reduced to NADPH, which subsequently generates one mole of reduced substrate.

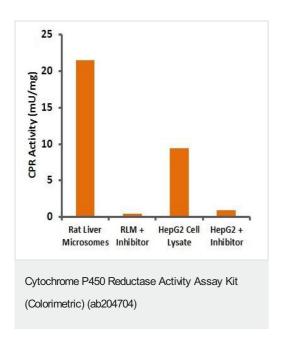




rat microsomal CPR (with and without inhibitor).

Reaction kinetics of recombinant human CPR (positive control) and

Cytochrome P450 Reductase Activity Assay Kit (Colorimetric) (ab204704)



Relative CPR activity detected in rat liver microsomes (RLM, 25 μg total protein) and HepG2 cell lysate (40 μg total protein).

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