

DetectX[®]

P450 Fluorescent 2 Plate Activity Kit

Catalog Number K011-F1



ARBOR
ASSAYS

FEATURES

- ▶ P450 activity without additions to your P450 reactions!
- ▶ Run your standard P450 drug:drug reaction - add ready-to-use formaldehyde sensor for signal
- ▶ Fluorescent plate-based readout, Exc=450nm, Em=510nm, 2 by 96-well plates

INTRODUCTION

The DetectX[®] P450 Activity kit is designed to measure the enzymatic activity of demethylation processes carried out by Cytochrome P450 (P450). The kit is unique in that the fluorescent substrate is not involved in the multi component P450 reaction, but measures the product of the demethylation, formaldehyde. The kit has been validated for a specific P450 system and should work with any P450 system that via demethylation is producing formaldehyde as a product. The formaldehyde substrate is added **after** the P450 reaction has been terminated, and the measured formaldehyde is then read at 510 nm with excitation at 450 nm.

KIT COMPONENTS

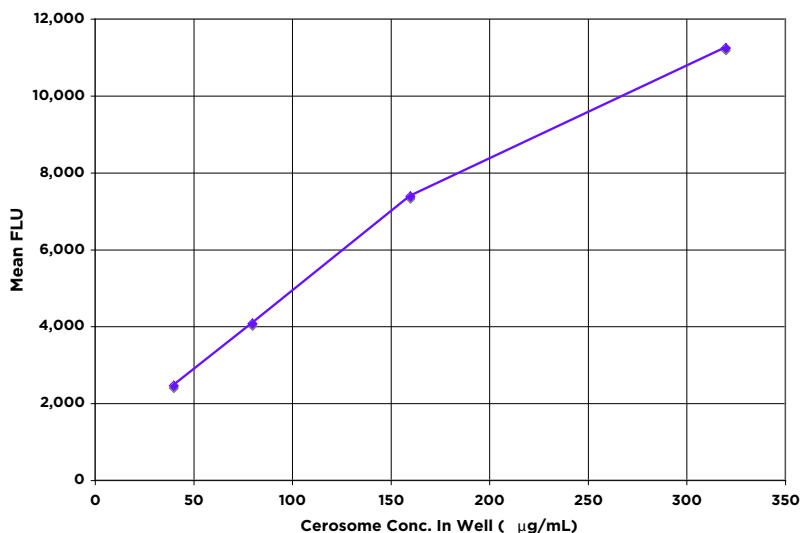
The kit provides an optimized buffer for 2B4 P450, NADPH, a stable formaldehyde standard, the Formaldehyde Detection Reagent (FDR) and a suitable 96 well plate for detecting the generated fluorescent signal. The end user will have to provide the P450 enzyme system or microsomal preparations necessary for activity, along with any inhibitors or activators. The end user should carry out the P450 reaction in our supplied reaction buffer or similar using optimized conditions for the reaction.

ASSAY PROTOCOL

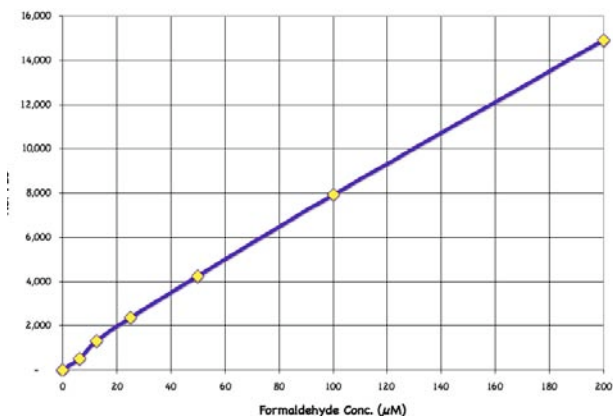
- Pipet 15 μ L of P450, oxidoreductase and cyt *b5* in pre-sonicated DLPC into duplicate wells.
- Add Assay Buffer and 5 μ L P450 drug substrates
- Incubate at 37°C for 5 minutes prior to addition of 5 μ L of NADPH.
- Incubate at 37°C for 15 minutes prior to addition of 5 μ L of Stop Solution followed by 25 μ L of the Formaldehyde Detection Reagent to each well.
- Incubate at 37°C for 30 minutes.
- Read activity in a fluorescent plate reader at 510 nm (Exc=450nm).

TYPICAL DATA

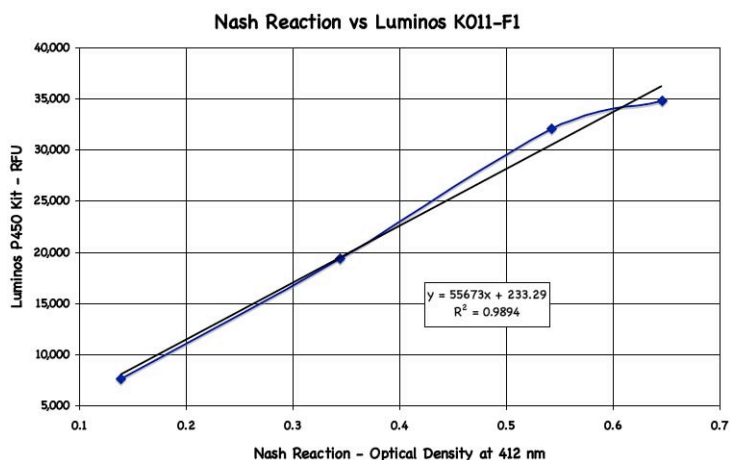
2B6 + Benzphetamine



CALIBRATE TO FORMALDEHYDE
Formaldehyde Calibration Curve



COMPARISON TO THE NASH METHOD
DetectX® K011-F1 Uses 1/2 the P450 enzymes



The cytochrome P450s (P450s) are a superfamily of heme containing enzymes that display tremendous diversity with regard to substrate specificity and catalytic activity. P450s use a plethora of both exogenous and endogenous compounds as substrates in enzymatic reactions. Usually they form part of multi component electron transfer reactions (see below). Catalysis by the eukaryotic P450 enzymes involves a multistep reaction cycle that includes two steps in which electron transfer is accomplished from a redox partner. The diflavin protein, NADPH cytochrome P450 reductase (reductase) contains both FAD and FMN and can transfer both electrons needed for the catalytic cycle. In some P450 reactions, the second electron of the reaction cycle also can be delivered by cytochrome b5. The P450 enzymes and cofactors of the mammalian drug-metabolizing system are embedded in the membrane of the endoplasmic reticulum. The P450s play a crucial role in the development of new drug entities as drug-drug interactions commonly inhibit cytochrome P450 activities.