

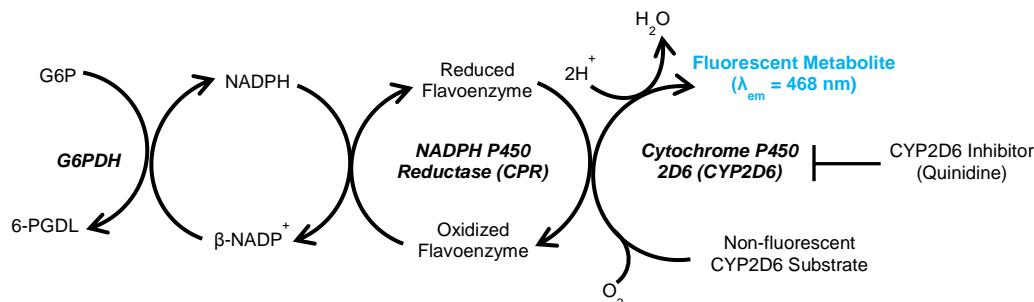
Cytochrome P450 2D6 (CYP2D6) Activity Assay Kit (Fluorometric)

7/15

(Catalog # K703-200; 200 Reactions; Store at -20°C)

I. Introduction:

Cytochrome P450 2D6 (CYP2D6, EC 1.14.14.1) is a member of the cytochrome P450 monooxidase (CYP) family of microsomal xenobiotic metabolism enzymes. CYPs are membrane-bound heme proteins responsible for Phase I biotransformation reactions, in which lipophilic drugs and other xenobiotic compounds are converted to more hydrophilic products to facilitate excretion from the body. CYP2D6 catalyzes oxidation of lipophilic bases with an aromatic ring and a nitrogen atom and is highly expressed in liver and brain tissue. The enzyme is responsible for metabolism of nearly 25% of all small molecule drugs commonly used by humans, particularly psychiatric drugs such as antidepressants, antipsychotics and stimulants. The CYP2D6 gene is highly polymorphic in the human population, with CYP2D6 activity ranging from complete metabolic deficiency to ultra-rapid metabolism. Due to this wide phenotypic variability, CYP2D6 is frequently implicated in drug toxicity and clinical drug/drug interactions. In addition, for drugs whose pharmacological activity requires metabolism from a pro-drug form, CYP2D6 inhibition or allelic deficiency can lead to decreased drug efficacy. BioVision's CYP2D6 Activity Assay Kit enables rapid measurement of native or recombinant CYP2D6 activity in biological samples such as liver microsomes. The assay utilizes a non-fluorescent CYP2D6-selective substrate that is converted into a highly fluorescent metabolite detected in the visible range (Ex/Em = 390/468 nm), ensuring a high signal-to-background ratio with little interference by autofluorescence. CYP2D6 specific activity is calculated by running parallel reactions in the presence and absence of the potent CYP2D6-selective inhibitor quinidine and subtracting any residual activity detected with the inhibitor present. The kit contains a complete set of reagents sufficient for performing 100 sets of paired reactions (in the presence and absence of inhibitor).



II. Applications:

- Rapid assessment of native/recombinant CYP2D6 activity in fractions prepared from tissues and cells.
- Screening of drugs and novel ligands for interaction with native/recombinant CYP2D6.

III. Sample Type:

- Human liver microsomes and liver S9 fractions
- Lysates of tissues and cultured cells, primary hepatocytes
- Heterologously expressed recombinant CYP2D6 preparations

IV. Kit Contents:

Components	K703-200	Cap Code	Part Number
CYP2D6 Assay Buffer	100 ml	NM	K703-200-1
AHMC Standard	1 vial	Yellow	K703-200-2
CYP2D6 Inhibitor (Quinidine)	1 vial	Amber	K703-200-3
NADPH Generating System (100X)	1 vial	Green	K703-200-4
β-NADP ⁺ Stock (100X)	1 vial	Blue	K703-200-5
CYP2D6 Substrate	1 vial	Red	K703-200-6
Recombinant Human CYP2D6	1 vial	Violet	K703-200-7

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) acetonitrile and DMSO
- Opaque white 96-well plates with flat bottom

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the CYP2D6 Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **AHMC Standard:** Reconstitute in 110 µl of DMSO and vortex until fully dissolved to yield a 2 mM stock solution. The AHMC stock solution should be stored at -20°C and is stable for at least 3 freeze/thaw cycles.
- **CYP2D6 Inhibitor (Quinidine):** Reconstitute in 220 µl of acetonitrile and vortex until fully dissolved to yield a 2 mM stock solution. The stock solution is stable for 2 months at -20°C. To obtain a 15 µM working solution of quinidine (5X final concentration), add 15 µl of the 2 mM stock solution to 1985 µl of CYP2D6 Assay Buffer. Store the 15 µM quinidine solution at -20°C and use within one week.
- **NADPH Generating System (100X):** Reconstitute with 440 µl CYP2D6 Assay Buffer, aliquot and store at -20°C. Avoid repeated freeze/thaw cycles and keep on ice while in use.

