

APD302Hu01 100μg Active Cytochrome P450 2D6 (CYP2D6)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Leu236~Gln472 Tags: N-terminal His-tag

Purity: >95%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl

and 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 5.9

Predicted Molecular Mass: 28.1kDa

Accurate Molecular Mass: 27kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

LAGKV LRFQKAFLTQ

LDELLTEHRM TWDPAQPPRD LTEAFLAEME KAKGNPESSF NDENLRIVVA DLFSAGMVTT STTLAWGLLL MILHPDVQRR VQQEIDDVIG QVRRPEMGDQ AHMPYTTAVI HEVQRFGDIV PLGVTHMTSR DIEVQGFRIP KGTTLITNLS SVLKDEAVWE KPFRFHPEHF LDAQGHFVKP EAFLPFSAGR RACLGEPLAR MELFLFFTSL LOHFSFSVPT GO

[ACTIVITY]

Cytochrome P450 2D6 (CYP2D6), a member of the cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body. In particular, CYP2D6 is responsible for the metabolism and elimination of approximately 25% of clinically used drugs, via the addition or removal of certain functional groups-specifically, hydroxylation, demethylation, and dealkylation. CYP2D6 is primarily expressed in the liver. It is also highly expressed in areas of the central nervous system, including the substantia nigra. This enzyme also metabolizes several endogenous substances, such as hydroxytryptamines, neurosteroids, and both m-tyramine and p-tyramine which CYP2D6 metabolizes into dopamine in the brain and liver. Besides, Cytochrome P450 Reductase (CPR) has been identified as an interactor of CYP2D6, thus a binding ELISA assay was conducted to detect the interaction of recombinant human VDR and recombinant human CPR. Briefly, CYP2D6 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to CPR-coated microtiter wells and incubated for 2h at 37°C.

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Wells were washed with PBST and incubated for 1h with anti-CYP2D6 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}\text{C}$. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of CYP2D6 and CPR was shown in Figure 1, and this effect was in a dose dependent manner.

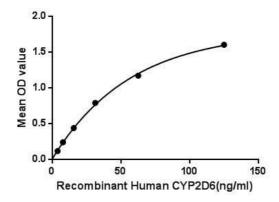


Figure 1. The binding activity of CYP2D6 with CPR.

[IDENTIFICATION]

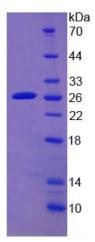


Figure 2. SDS-PAGE

Sample: Active recombinant CYP2D6, Human

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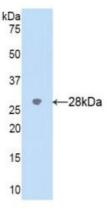


Figure 3. Western Blot

Sample: Recombinant CYP2D6, Human;

Antibody: Rabbit Anti-Human CYP2D6 Ab (PAD302Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.