

APD294Hu01 100µg

Active Cytochrome P450 1A2 (CYP1A2)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Ala2~Ser231

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >95%

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: 8.0

Predicted Molecular Mass: 55.2kDa

Accurate Molecular Mass: 55kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

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]

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ALSQSVPFSAATELLLASAIFCLVFWVLKGLRPRVPKGLKSPPEPWGWPL
LGHVLTGKNPHLALSRMSQRYGDVLQIRIGSTPVLVLSRLDIRQALVLR
QGDDFKGRPDLYTSTLITDQSLTFSTDSGPVWAARRRLAQNALNTFSIA
SDPASSSSCYLEEHSKEAKALISRLQELMAGPGHFDYPNQVVSVANVI
GAMCFGQHFESSDEMLSLVKNTHEFVETAS
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[ACTIVITY]

CYP1A2 (Cytochrome P450 1A2) belongs to the group of proteins which contains heme as a cofactor. CYP1A2 oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. Besides, ASAH1 (Acid ceramidase) has been identified as an interactor of CYP1A2 through affinity capture-MS. Thus a binding ELISA assay was conducted to detect the interaction of recombinant human CYP1A2 and recombinant human ASAH1. Briefly, CYP1A2 were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL were then transferred to ASAH1-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CYP1A2 mAb, 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of CYP1A2 and ASAH1 was shown in Figure 1, and this effect was in a dose dependent manner.

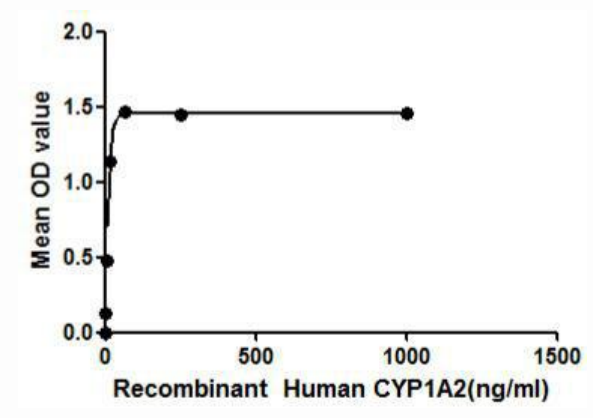


Figure 1. The binding activity of CYP1A2 with ASAH1.

[IDENTIFICATION]

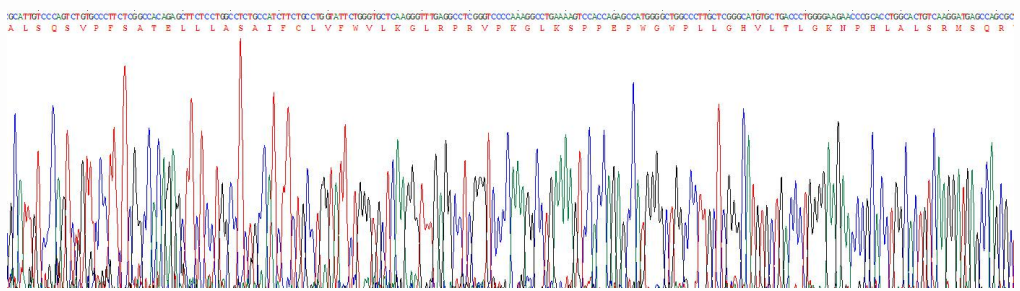


Figure 2. Gene Sequencing (extract)

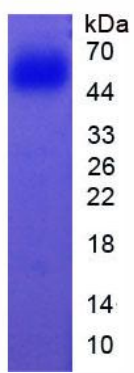


Figure 3. SDS-PAGE

Sample: Active recombinant CYP1A2, Human

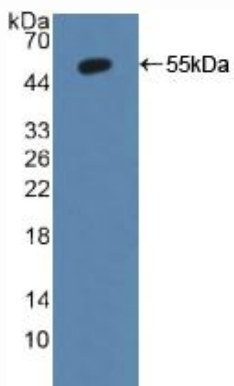


Figure 4. Western Blot

Sample: Recombinant CYP1A2, Human;

Antibody: Rabbit Anti-Human CYP1A2 Ab (PAD294Hu01)