

APD297Hu01 100µg Active Cytochrome P450 1B1 (CYP1B1) Organism Species: Homo sapiens (Human)

Instruction manual

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

1st Edition (Apr. 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Asp374~Phe516 Tags: N-terminal His-tag

Purity: >98%

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: 7.9

Predicted Molecular Mass: 17.6kDa

Accurate Molecular Mass: 18&16kDa as determined by SDS-PAGE reducing

conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
- 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
- 5. Polymerization of the target protein: Dimerization, multimerization etc.



[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]

DQPNLPY VLAFLYEAMR FSSFVPVTIP
HATTANTSVL GYHIPKDTVV FVNQWSVNHD PLKWPNPENF DPARFLDKDG
LINKDLTSRV MIFSVGKRRC IGEELSKMQL FLFISILAHQ CDFRANPNEP
AKMNFSYGLT IKPKSF

[ACTIVITY]

Cytochrome P450 family 1 subfamily B member 1 (CYP1B1) is an enzyme, Which belongs to the cytochrome P450 superfamily. CYP1B1 can catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. Besides, Heat Shock Protein family B (small) member 2 (HSPb2) has been identified as an interactor of CYP1B1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CYP1B1 and recombinant human HSPb2, CYP1B1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSPb2-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and

incubated for 1h with anti-CYP1B1 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of CYP1B1 and HSPb2 was shown in Figure 1, and this effect was in a dose dependent manner.

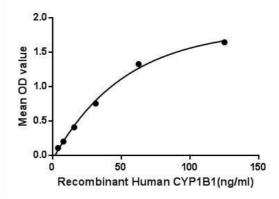


Figure 1. The binding activity of CYP1B1 with HSPb2.

[IDENTIFICATION]

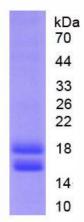


Figure 2. SDS-PAGE

Sample: Active recombinant CYP1B1, Human

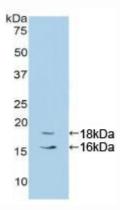


Figure 3. Western Blot

Sample: Recombinant CYP1B1, Human;

Antibody: Rabbit Anti-Human CYP1B1 Ab (PAD297Hu01)