

APD299Hu01 100µg
Active Active Cytochrome P450 3A4 (CYP3A4)
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: Pro344~Asp497

Tags: N-terminal His-tag

Purity: >90%

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: 9.4

Predicted Molecular Mass: 21.6kDa

Accurate Molecular Mass: 22kDa 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]

PPTYDTV
LQMEYLDMMV NETLRLFPIA MRLERVCKKD VEINGMFIPK GVVVMIPSYA
LHRDPKYWTE PEKFLPERFS KKNKDNIDPY IYTPFGSGPR NCIGMRFALM
NMKLALIRVL QNFSFKPCKE TQIPLKLSLG GLLQPEKPVV LKVESRD

[ACTIVITY]

Cytochrome P450 3A4 (CYP3A4) is an important enzyme in the body, mainly found in the liver and in the intestine. It oxidizes small foreign organic molecules (xenobiotics), such as toxins or drugs, so that they can be removed from the body. CYP3A4 is a member of the cytochrome P450 family of oxidizing enzymes and the CYP3A4 is the most common and the most versatile one in drug metabolism. Besides, Heat Shock 70kDa Protein 8 (HSPA8) has been identified as an interactor of CYP3A4, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CYP3A4 and recombinant human HSPA8. Briefly, CYP3A4 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSPA8-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CYP3A4 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 CYP3A4 and HSPA8 was shown in Figure 1, and this effect was in a dose dependent manner.

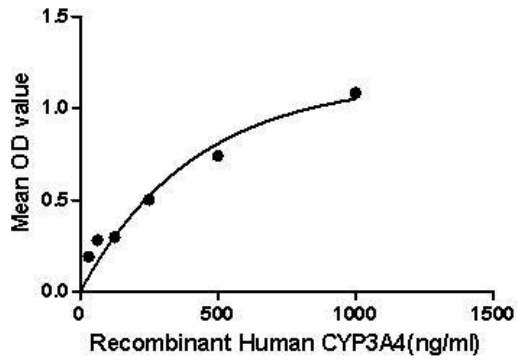


Figure 1. The binding activity of CYP3A4 with HSPA8.

[IDENTIFICATION]

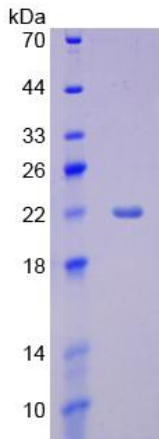


Figure 2. SDS-PAGE

Sample: Active recombinant CYP3A4, Human

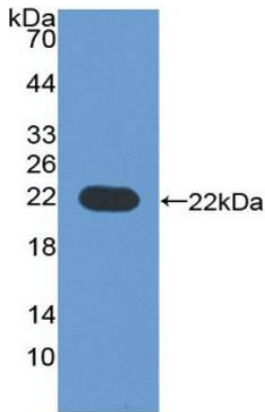


Figure 3. Western Blot

Sample: Recombinant CYP3A4, Human;

Antibody: Rabbit Anti-Human CYP3A4 Ab (PAD299Hu01)

[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.