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APD295Ra01 100µg Active Cytochrome P450 1A1 (CYP1A1) Organism Species: Rattus norvegicus (Rat) *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: Ser251~His521

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: 6.8

Predicted Molecular Mass: 61.5kDa

Accurate Molecular Mass: 62kDa 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.

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**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]

SLDAFKDLNK KFYSFMKKLI KEHYRTFEKG HIRDITDSLI EHCQDRRLDE NANVQLSDDK VITIVFDLFG AGFDTITTAI SWSLMYLVTN PRIQRKIQEE LDTVIGRDRQ PRLSDRPQLP YLEAFILETF RHSSFVPFTI PHSTIRDTSL NGFYIPKGHC VFVNQWQVNH DQELWGDPNE FRPERFLTSS GTLDKHLSEK VILFGLGKRK CIGETIGRLE VFLFLAILLQ QMEFNVSPGE KVDMTPAYGL TLKHARCEHF QVQMRSSGPQ H

#### [ACTIVITY]

Cytochrome P450 1A1 (CYP1A1) is a member of Cytochromes P450 superfamily of enzymes. Cytochromes P450 are a group of heme-thiolate monooxygenases. It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. CYP1A1 is also known as AHH (aryl hydrocarbon hydroxylase). It is involved in the metabolic activation of aromatic hydrocarbons (polycyclic aromatic hydrocarbons, PAH). Besides, Heat Shock 70kDa Protein 4 (HSPA4) has been identified as an interactor of CYP1A1, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat CYP1A1 and recombinant rat HSPA4. Briefly, CYP1A1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSPA4-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CYP1A1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were incubated 15-25 minutes at 37°C. Finally, add 50µL stop solution to the wells and read at

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450nm immediately. The binding activity of of CYP1A1 and HSPA4 was shown in Figure 1, and this effect was in a dose dependent manner.

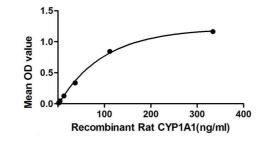
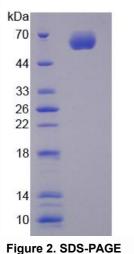


Figure 1. The binding activity of CYP1A1 with HSPA4



#### [IDENTIFICATION]

Sample: Active recombinant CYP1A1, Rat

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kDa 70	←62kDa
44	100 - 1000 000 00 00 00 00 00 00 00 00 00 00
33	
26	
22	
18	
14	
10	

Figure 3. Western Blot

Sample: Recombinant CYP1A1, Rat;

Antibody: Rabbit Anti-Rat CYP1A1 Ab (PAD295Ra01)