**APG357Hu01 100μg**
**Active Cholesterol-25-Hydroxylase (CH25H)**
**Organism Species: *Homo sapiens* (Human)**
***Instruction manual***

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

# [ PROPERTIES ]

**Source:** Prokaryotic expression.
**Host:** *E. coli*
**Residues:** Trp142~Arg272
**Tags:** His and TrxA Tag
**Purity:** >95%
**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method).
**Buffer Formulation:** PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .
**Original Concentration:** 200μg/mL
**Applications:** Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
**Predicted isoelectric point:** 8.3
**Predicted Molecular Mass:** 35.9kDa
**Accurate Molecular Mass:** 31kDa 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 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

## [ STORAGE AND STABILITY ]

**Storage:** Avoid repeated freeze/thaw cycles.

Store at 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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 ]

WHLLHHKVPWLYRTFHKVHHQNSSSFALATQYMSVWELFSLGFFDMMNVTLLGCHPLTTLTFHVVNIWLSVEDHSGYNFPWSTHRLVPF
GWYGGVVHHDLHHSHFNCNFAPYFTHWDKILGTLRTASVPAR

## [ ACTIVITY ]

Cholesterol-25-Hydroxylase (CH25H) is an endoplasmic reticulum-associated enzyme that catalyzes the hydroxylation of cholesterol at the 25-position to produce 25-hydroxycholesterol (25-HC). This oxysterol plays critical roles in lipid metabolism, immune regulation, and antiviral responses by modulating the activity of sterol-responsive transcription factors such as SREBP and LXR. CH25H is highly induced by inflammatory signals, particularly interferons, linking it to innate immunity and cholesterol homeostasis. Its product, 25-HC, also influences membrane properties and suppresses viral entry. CH25H interacts with Cytochrome P450 7A1 (CYP7A1), the rate-limiting enzyme in bile acid synthesis, by producing 25-HC to inhibit CYP7A1 activity, thereby linking cholesterol oxidation to bile acid regulation. CH25H-derived 25-HC binds and inhibits CYP7A1 to suppress bile acid production. Thus a functional binding ELISA assay

was conducted to detect the interaction of recombinant human CH25H and recombinant mouse CYP7A1 . Briefly, biotin-linked CH25H were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 μl were then transferred to CYP7A1-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μl stop solution to the wells and read at 450nm immediately. The binding activity of CH25H and CYP7A1 was shown in Figure 1, the EC50 for this effect is 0.139μg/mL.

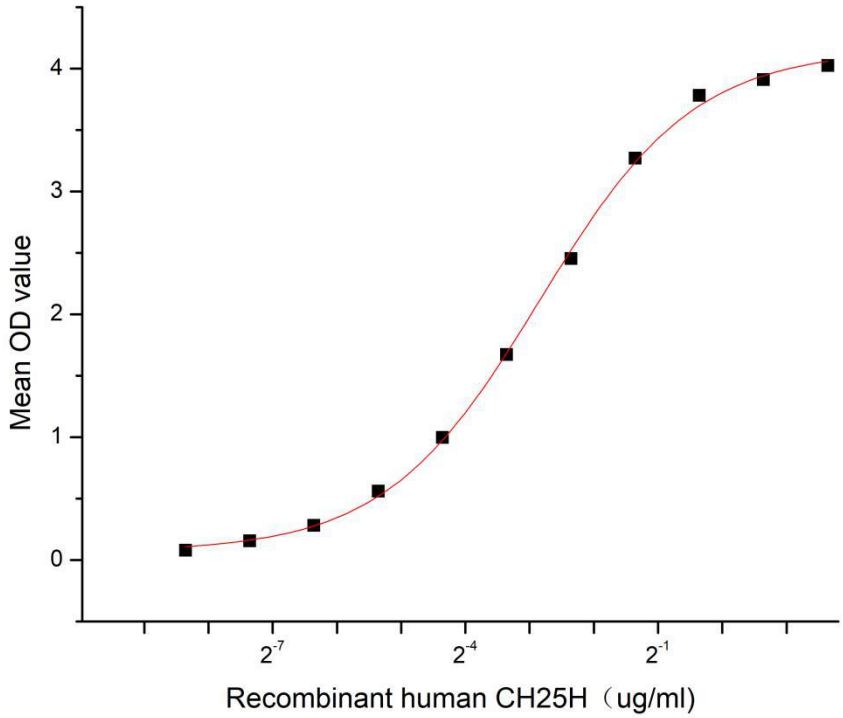


**Figure 1. The binding activity of recombinant human CH25H and recombinant mouse CYP7A1**

# [ IDENTIFICATION ]

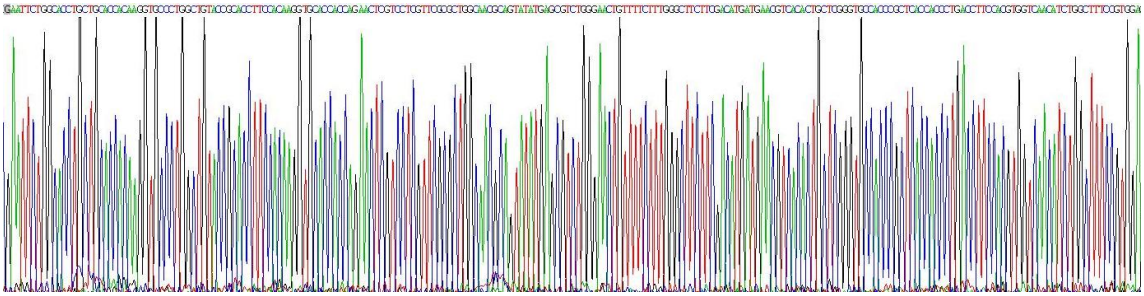

**Figure 2. Gene Sequencing (extract)**

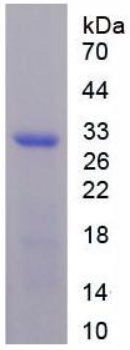


**Figure 3. SDS-PAGE**

**Sample: Active recombinant CH25H, Human**

# [ IMPORTANT NOTE ]

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