

APF173Hu01 100µg

Active 17-Beta-Hydroxysteroid Dehydrogenase Type 3 (HSD17b3)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

### [PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Met1~Arg310
Tags: N-terminal His-tag

**Purity: >90%** 

**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .

Original Concentration: 200µg/mL

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 8.9

Predicted Molecular Mass: 38.2kDa

Accurate Molecular Mass: 33kDa 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 2-8°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]

MGDVLEQFFI LTGLLVCLAC LAKCVRFSRC VLLNYWKVLP KSFLRSMGQW AVITGAGDGI GKAYSFELAK RGLNVVLISR TLEKLEAIAT EIERTTGRSV KIIQADFTKD DIYEHIKEKL AGLEIGILVN NVGMLPNLLP SHFLNAPDEI QSLIHCNITS VVKMTQLILK HMESRQKGLI LNISSGIALF PWPLYSMYSA SKAFVCAFSK ALQEEYKAKE VIIQVLTPYA VSTAMTKYLN TNVITKTADE FVKESLNYVT IGGETCGCLA HEILAGFLSL IPAWAFYSGA FQRLLLTHYV AYLKLNTKVR

#### [ACTIVITY]

17-Beta-Hydroxysteroid Dehydrogenase Type 3 (HSD17b3) belongs to the HSD17B family with NAD(P)H/NAD(P)+-dependent oxidoreductase activity that catalyzes the interconversion between 17-ketosteroids and 17-hydroxysteroids to maintain the balance between less potent (17-keto) and more potent (17  $\beta$ -hydroxy) forms of estrogens and androgens. This isoform of 17 beta-hydroxysteroid dehydrogenase is expressed predominantly in the testis and catalyzes the conversion of androstenedione to testosterone.

3-oxo-5-alpha-steroid 4-dehydrogenase 2 (SRD5a2 ) is a enzyme which plays a central role in sexual differentiation and androgen physiology. A functional ELISA assay was conducted to detect the interaction of recombinant human HSD17b3 and recombinant human SRD5a2. Briefly, HSD17b3 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ l were then transferred to SRD5a2-coated microtiter wells and incubated for 1h at  $37^{\circ}\!\!\mathrm{C}$ . Wells were washed with PBST and incubated for 1h with anti-HSD17b3 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at  $37^{\circ}\!\!\mathrm{C}$ , wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at  $37^{\circ}\!\!\mathrm{C}$ . Finally, add 50  $\mu$ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant human HSD17b3 and recombinant human SRD5a2 was shown in Figure 1, the EC50 for this effect is 27.6 ng/mL.

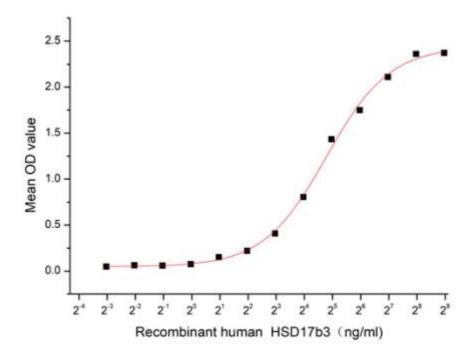


Figure 1. The binding activity of recombinant human HSD17b3 and human SRD5a2

# [IDENTIFICATION]

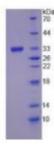


Figure 2. SDS-PAGE

Sample: Active recombinant HSD17b3, 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.