APH430Hu01 100μg
Active Pyruvate Carboxylase (PC)
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: Pro36~Glu486
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: 6.2
Predicted Molecular Mass: 53.6kDa
Accurate Molecular Mass: 54kDa as determined by SDS-PAGE reducing conditions.

[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]

PIKKV MVANRGEIAI
RVFRACTELG IRTVAIYSEQ DTGQMHRQKA DEAYLIGRGL APVQAYLHIP
DIIKVAKENN VDAVHPGYGF LSERADFAQA CQDAGVRFIG PSPEVVRKMG
DKVEARAIAI AAGVPVVPGT DAPITSLHEA HEFSNTYGFP IIFKAAYGGG
GRGMRVVHSY EELEENYTRA YSEALAAFGN GALFVEKFIE KPRHIEVQIL
GDQYGNILHL YERDCSIQRR HQKVVEIAPA AHLDPQLRTR LTSDSVKLAK
QVGYENAGTV EFLVDRHGKH YFIEVNSRLQ VEHTVTEEIT DVDLVHAQIH
VAEGRSLPDL GLRQENIRIN GCAIQCRVTT EDPARSFQPD TGRIEVFRSG
EGMGIRLDNA SAFQGAVISP HYDSLLVKVI AHGKDHPTAA TKMSRALAEF
RVRGVKTNIA FLQNVLNNQQ FLAGTVDTQF IDENPE

[ACTIVITY]

Pyruvate carboxylase (PC) is a mitochondrial, biotin-containing enzyme catalyzing the ATP-dependent synthesis of oxaloacetate from pyruvate and bicarbonate, with a critical anaplerotic role in sustaining the brain metabolism. Pyruvate carboxylase (PC) deficiency is a rare autosomal recessive mitochondrial neurometabolic disorder of energy deficit resulting in high morbidity and mortality, with limited therapeutic options. Besides, PCK2 has been identified as an interactor of PC, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human PC and recombinant human PCK2. Briefly, biotin-linked PC were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to PCK2-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30 min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 μl stop solution to the wells and read at 450 nm immediately.

The binding activity of PC and PCK2 was shown in Figure 1, the EC50 for this effect is 0.056 ug/mL.

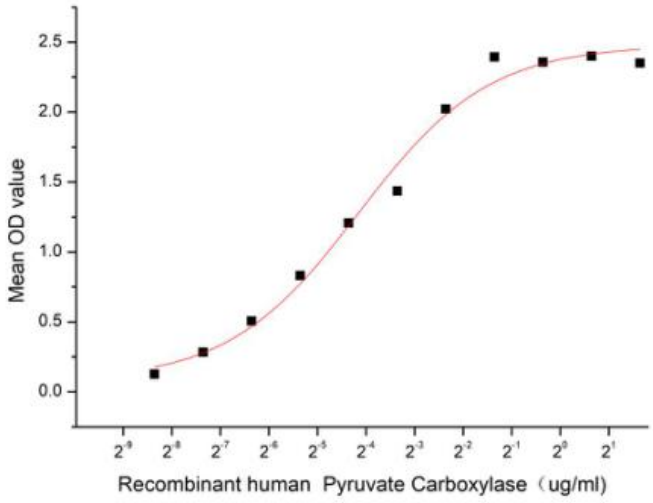

Figure 1. The binding activity of recombinant human PC and recombinant human PCK2

[IDENTIFICATION]

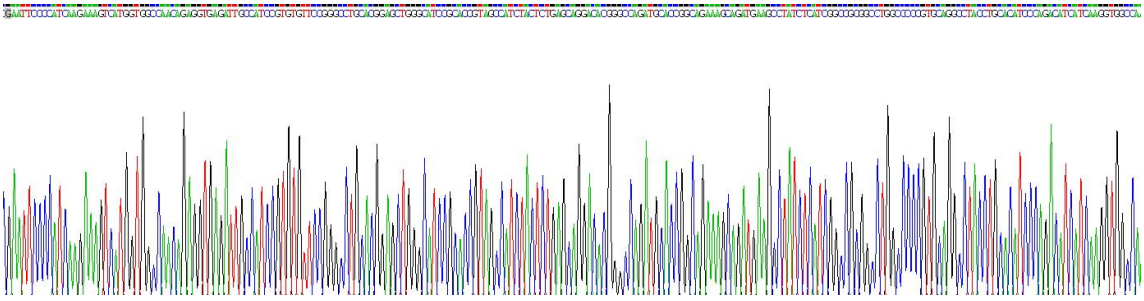

Figure 2. Gene Sequencing (extract)

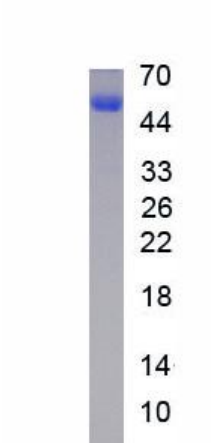

Figure 3. SDS-PAGE

Sample: Active recombinant PC, 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.