

**APE848Hu01 100µg**  
**Active Diacylglycerol Kinase Epsilon (DGKe)**  
**Organism Species: Homo sapiens (Human)**  
***Instruction manual***

FOR IN VITRO USE AND RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Leu243~Gly502

**Tags:** N-terminal His-tag

**Purity:** >95%

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

**Applications:** Cell culture; Activity Assays; In vivo assays.

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

**Predicted isoelectric point:** 7.7

**Predicted Molecular Mass:** 32.5kDa

**Accurate Molecular Mass:** 33kDa 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 ]**

```
LLNPVQVF
DVTKTPIKA LQLCTLLPYY SARVLVCGGD GTVGWVLDV DDMKIKGQEK
YIPQVAVLPL GTGNDLSNTL GWGTGYAGEI PVAQVLRNVM EADGIKLDWR
KVQVTNKGYY NLRKPKEFTM NNYFSVGPDA LMALNFHAHR EKAPSLFSSR
ILNKAVYLFY GTKDCLVQEC KDLNKKVELE LDGERVALPS LEGIIVLNIG
YWGGGCRLEW GMGDETYPLA RHDDGLLEV VGVYGSFHCAQ IQVKLANPFR
IG
```

## **[ ACTIVITY ]**

DGKe (Diacylglycerol kinase epsilon) is an enzyme that mainly involved in the regeneration of phosphatidylinositol (PI) from diacylglycerol in the PI-cycle during cell signal transduction. PDHA1 (Pyruvate dehydrogenase E1 component subunit alpha) has been proven to be an interactor of DGKe through Affinity Capture-MS. Thus a binding ELISA assay was conducted to detect the interaction of recombinant human DGKe and recombinant human PDHA. Briefly, DGKe were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL DGKe were then transferred to PDHA-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-DGKe 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 DGKe and PDHA was shown in Figure 1, and this effect was in a dose dependent manner.

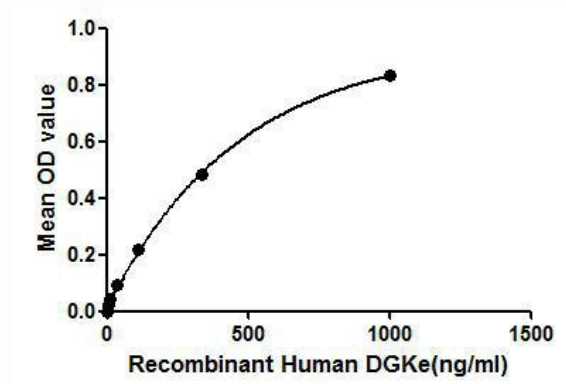


Figure 1. The binding activity of DGKe with PDHA.

## [ IDENTIFICATION ]

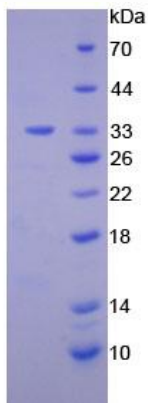
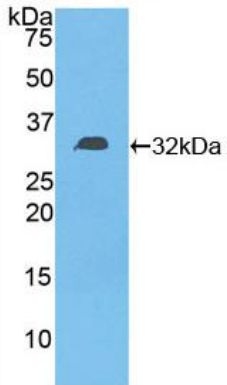


Figure 2. SDS-PAGE

Sample: Active recombinant DGKe, Human



**Figure 3. Western Blot**

**Sample: Recombinant DGKe, Human;**

**Antibody: Rabbit Anti-Human DGKe Ab (PAE848Hu01)**