

APC601Hu01 100µg
Active Valosin Containing Protein (VCP)
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: Gly125~Ile371

Tags: N-terminal His-tag

Purity: >95%

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: 5.9

Predicted Molecular Mass: 31.2kDa

Accurate Molecular Mass: 36kDa 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 2-8°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.

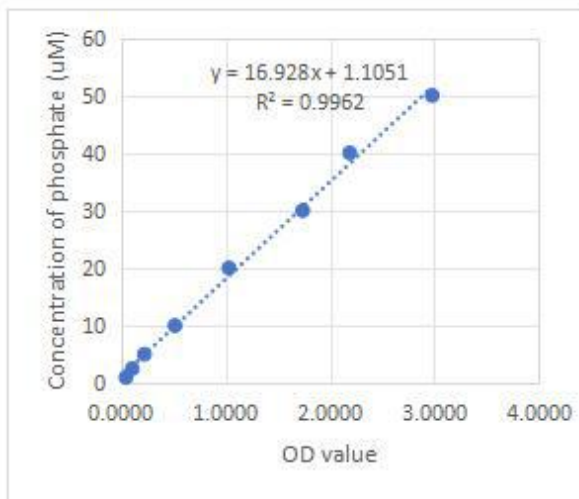
[**SEQUENCE**]

```
GITGNL FEVYLKPYFL EAYRPIRKGD
IFLVRGGMRA VEFKVVETDP SPYCIVAPDT VIHCEGEPIK REDEEESLNE
VGYDDIGGCR KQLAQIKEMV ELPLRHPALF KAIGVKPPRG ILLYGPPGTG
KTLIARAVAN ETGAFFFLIN GPEIMSKLAG ESESNLRKAF EEAEKNAPI
IFIDELDAIA PKREKTHGEV ERRIVSQLLT LMDGLKQRAH VIVMAATNRP
NSIDPALRRF GRFDREVDIG I
```

[**ACTIVITY**]

VCP is an ATPase Associated with diverse cellular Activities (AAA+) that has a key role in maintaining cellular homeostasis. VCP is ubiquitously expressed in tissues. At cellular level, VCP functions in many cellular compartments; VCP localizes mainly in the cytoplasm, while a smaller fraction binds to organelles or localizes in the nucleus, where it is implicated in different pathways. VCP assembles and acts as a homo-hexamer, in which each monomer is structured in an N-terminal domain that interacts with adaptors and co-factors; in two ATPase domains, D1 and D2, that by hydrolyzing ATP concur, respectively, in the hexamer formation and in supporting VCP activity; and in a C-terminal domain that binds to a small subset of co-factors and adaptors cooperating with D2 activity. The main role of VCP is to recognize and to extract ubiquitinated proteins from membranes, protein complexes, protein aggregates, or chromatin, modulating their ubiquitination or de-ubiquitination, and to enhance their degradation through the UPS or the autophagic pathway. The activity of recombinant human VCP was measured by its ability to hydrolyze the substrate ATP to phosphate which was detected by the malachite green phosphate detection Kit (Beyotime # S0196M). The reaction was performed in the assay buffer 20 mM HEPES, 13 mM NaCl and

1% glycerol, pH 8.5, initiated by addition 27 μ L of various concentrations of VCP (diluted by assay buffer) to 3 μ L of 100 mM ATP and 100 mM MgCl₂ mixture. The final well serves as a negative control with no VCP, replaced with 27 μ L assay buffer. Incubated at 37 $^{\circ}$ C for 5min, then stop the reaction by quickly freezing samples in the dry ice and ethanol. 4 μ l samples was added to 196 μ l assay buffer after thawing at room temperature followed by adding 70 μ l phosphate detection agent and incubated at room temperature for 30min, then read at a wavelength of 630 nm. The specific activity of recombinant human VCP is >1000 pmol/min/ μ g.



OD(630nm)	Phosphate (uM)
2.9771	50
2.1898	40
1.7402	30
1.0328	20
0.5177	10
0.2256	5
0.1086	2.5
0.0492	1

Figure 1. The standard curve of Phosphate

[IDENTIFICATION]

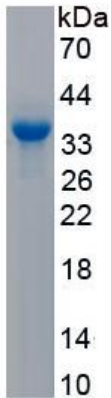


Figure 2. SDS-PAGE

Sample: Active recombinant VCP, 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.