

**APA873Hu61 100µg**

**Active Heat Shock Protein 70 (HSP70)**

**Organism Species: *Homo sapiens* (Human)**

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Met1~Asp641

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

**Purity:** >90%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**Buffer Formulation:** PBS, pH7.4, containing 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:** 5.3

**Predicted Molecular Mass:** 71.7kDa

**Accurate Molecular Mass:** 75kDa 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 ]**

```
MAKAAAIGIDLGTYSVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVALNPQNTVFDKRLIGRKFGDPVVQSDMK  
HWPFQVINDGDKPKVQVSYPGETKAFYPEEISSMVLTKMKEIAEAYLGYPVTNAVITVPAYFNDQRQATKDAGVIAGLNLRIINEP  
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FFNGRDLNKSINPDEAVAYGAAVQAAIILMGDKSENVQDLLLDVAPLSLGLTAGGVMTALIKRNSTIPTKQTQIFTTYSDNQPGVLI  
QVYEGERAMTKDNNLLGRFELSGIPPAPRGVPQIEVTFDIDANGILNVATDKSTGKANKITITNDKGRLSKEEIERMVQEAKEYKAE  
DEVQRRERSAKNALESYAFNMKSAVEDEGLKGKISEADKKKVLDKCQEVISWLDANTLAEKDEFEHKRKELEQVCNPIISGLYQGAGG  
PGPGGFGAQQPKGGSGSGPTIEEVD
```

## **[ ACTIVITY ]**

Heat Shock Protein 70 (HSP70) is a vital and highly conserved protein. It exists in organisms from bacteria to humans. Under normal conditions, it aids in protein folding and maintenance of cellular proteostasis. When cells encounter stress like heat or toxins, HSP70 expression surges. It then prevents protein aggregation, assists in refolding damaged proteins, and plays a key role in cell survival and stress response, safeguarding cellular function and integrity. Besides, The activation of HSP70 protein depends on the hydrolysis of ATP, which can produce inorganic Pi phosphate. Thus, the activity of recombinant human HSP70 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  $\mu$  L of various concentrations of HSP70 (diluted by assay buffer) to 3  $\mu$  L of 100 mM ATP and 100 mM MgCl<sub>2</sub> mixture. The final well serves as a negative control with no HSP70, replaced with 27  $\mu$  L assay buffer. Incubated at 37°C for 5min, then stop the reaction by quickly freezing samples in the dry ice and ethanol. 4  $\mu$  L samples was added to 196  $\mu$  L assay buffer after thawing at room temperature followed by adding 70  $\mu$  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

HSP70 is >12000 pmol/min/μg.

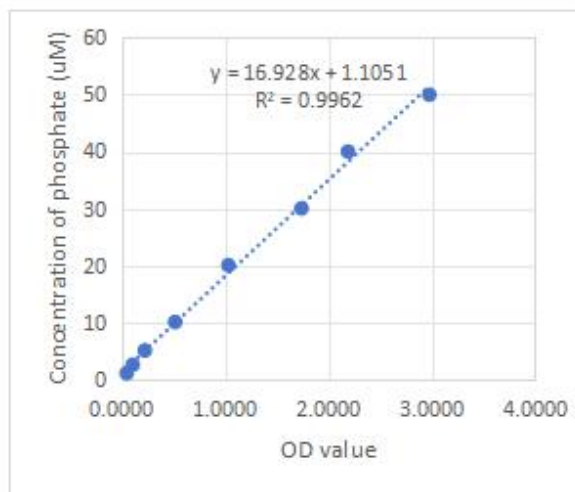


Figure 1. The standard curve of Phosphate

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

## [ IDENTIFICATION ]

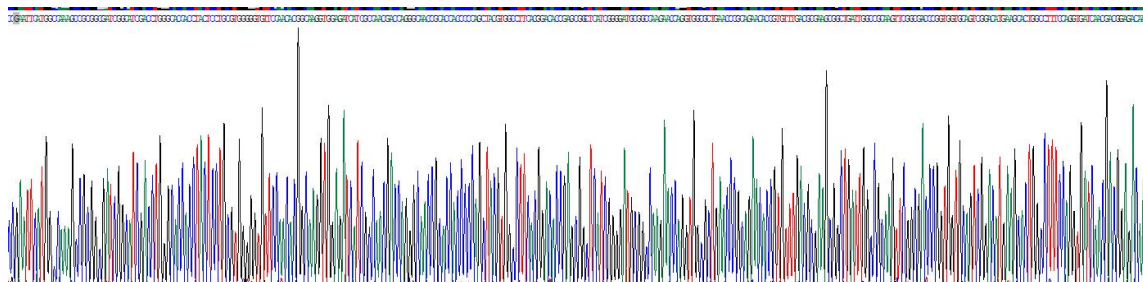
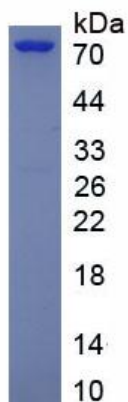


Figure 2. Gene Sequencing (extract)



**Figure 3. SDS-PAGE**

**Sample: Active recombinant HSP70, 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.