

**APB393Mu01 100µg**  
**Active Activating Transcription Factor 6 (ATF6)**  
**Organism Species: *Mus musculus* (Mouse)**  
***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:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Met1~Cys308

**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% Sarcosyl, 5%Trehalose .

**Original Concentration:** 200µg/mL

**Applications:** Activity Assays.

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

**Predicted isoelectric point:** 5.6

**Predicted Molecular Mass:** 36.7kDa

**Accurate Molecular Mass:** 45kDa 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.

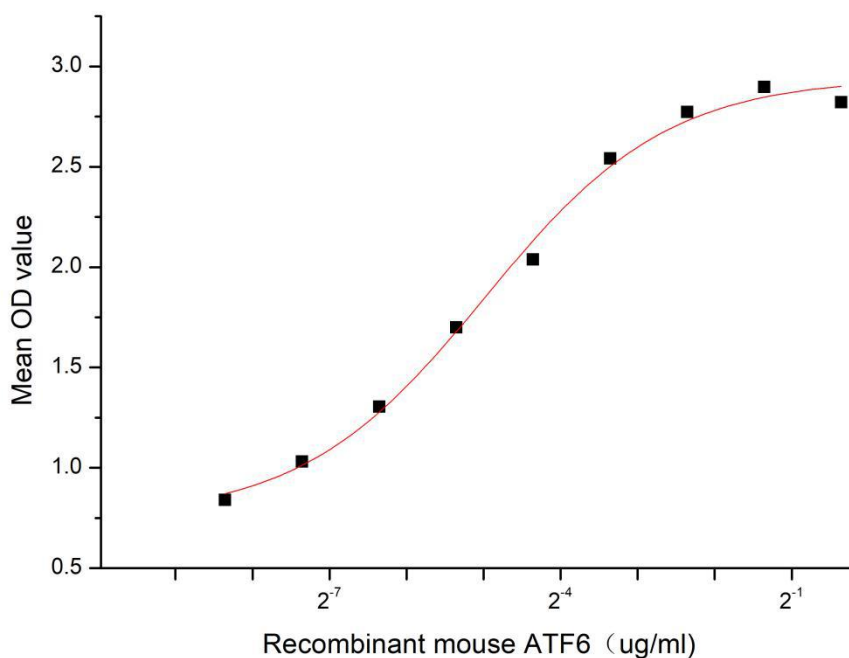
## **[ SEQUENCE ]**

MESPFSPVLPHGPDDEWESTLFAELGYFTDSTDVHFDAHEAYENNFHDHLNFDLDLMPWESD  
LWSPGSHFCSDMKAEPQPLSPASSSCSISSPRSTDSCSSTQHVPEELDLLSSSQSPLSLYGDSCNSP  
SSVEPLKEEKPVTGPGNKTEHGLTPKKKIQMSSKPSVQPKLLLPAAPKTQTNASVPAKAIITLP  
ALMPLAKQQSIISIQPAPTKGQTVLLSQPTVVQLQSPAVLSSAQPVLAVTGGAAQLPNHVVNVL  
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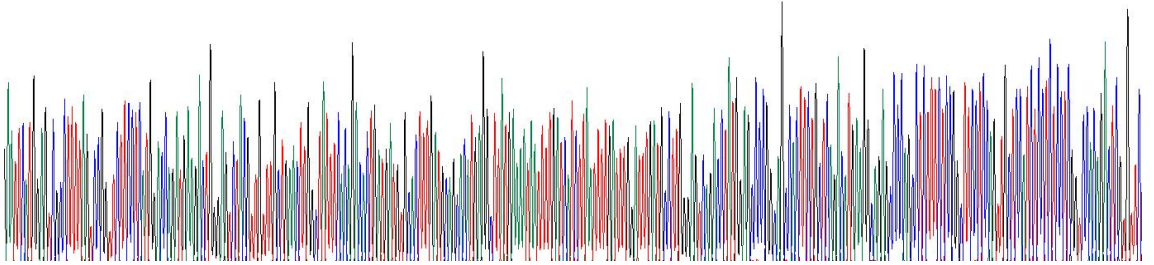
## **[ ACTIVITY ]**

Activating Transcription Factor 6 (ATF6) is a key protein in the endoplasmic reticulum (ER) stress response. It is a transmembrane protein located in the ER membrane. Under normal conditions, ATF6 is bound to Heat Shock 70kDa Protein 5 (HSPA5), also known as GRP78, and remains inactive. When ER stress occurs due to the accumulation of unfolded or misfolded proteins in the ER lumen, HSPA5 preferentially binds to these abnormal proteins, releasing ATF6. The released ATF6 then translocates to the Golgi apparatus, where it is cleaved by proteases. The cleaved ATF6 fragment enters the nucleus and activates the transcription of genes involved in the unfolded protein response, helping the cell to restore ER homeostasis. Thus a functional ELISA assay was conducted to detect the

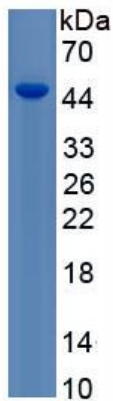
interaction of recombinant mouse ATF6 and recombinant mouse HSPA5. Briefly, ATF6 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ l were then transferred to HSPA5-coated microtiter wells and incubated for 1h at 37  $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-ATF6 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37  $^{\circ}$ C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37  $^{\circ}$ C. Finally, add 50  $\mu$ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant mouse ATF6 and recombinant mouse HSPA5 was shown in Figure 1, the EC<sub>50</sub> for this effect is 0.031ug/mL.



**Figure 1. The binding activity of recombinant mouse ATF6 and mouse HSPA5**

[illegible]

### Figure 2. Gene Sequencing (extract)



### Figure 3. SDS-PAGE

**Sample: Active recombinant ATF6, Mouse**

**[ 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.