

APB214Hu01 100µg
Active Aspartate Aminotransferase (AST)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Met1~Gln413

Tags: N-terminal His-tag

Purity: >97%

Traits: Freeze-dried powder

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 1000µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.6

Predicted Molecular Mass: 47.8kDa

Accurate Molecular Mass: 44kDa 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]

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MAPPSVFAEV PQAQPVLVFK LTADFREDPD PRKVNLGVGA YRTDDCHPWV
LPVVKKVEQK IANDNSLNHE YLPILGLAEF RSCASRLALG DDSPALKEKR
VGGVQSLGGT GALRIGADFL ARWYNGTNNK NTPVYVSSPT WENHNAVFS A
AGFKDIRSYR YWDAEKRGDL LQGFLNDLEN APEFSIVVLH ACAHNPTGID
PTPEQWKQIA SVMKHRFLFP FFDSAYQGFA SGNLERDAWA IRYFVSEGFE
FFCAQSF SKN FGLYNERVGN LTVVGKEPES ILQVLSQMEK IVRITWSNPP
AQGARIVAST LSNPELFE EW TGNVKT MADR ILTMRSELRA RLEALKTPGT
WNHITDQIGM FSFTGLNPKQ VEYLVNEKHI YLLPSGRINV SGLTTKNLDY
VATSIHEAVT KIQ
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[ACTIVITY]

Aspartate transaminase(AST) or aspartate aminotransferase, also known as AspAT/ASAT/AAT or glutamic oxaloacetic transaminase, is a pyridoxal phosphate-dependent transaminase enzyme AST catalyzes the reversible transfer of an α -amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. In this test, an amino group is transferred of from aspartate to α -ketoglutarate. The products of this reversible transamination reaction are oxaloacetate and glutamate. The oxaloacetic acid can be decomposed into pyruvate and carbon dioxide with the present of phenylamine citrate. The activity of aspartate transaminase can be measured by calculating the concentration of the pyruvate. The reaction was performed in adding 10 μ l different concentration recombinant AST(the blank tube add 10 μ l phosphate buffer) to 50 μ l mixture substrate containing 2mM 2-Ketoglutaric acid, 0.1M L-aspartic acid,

in 0.2M phosphate buffer,pH7.4, incubate at 37 °C for 1h, then add 10µl phenylamine citrate and 50µl 2,4-dinitrophenylhydrazine continue incubate at 37 °C for 20min, stop the action by adding 500µl 0.4M NaOH, read the OD value at 520nm. Standard curve prepare by double dilute 2µM pyruvate with phosphate bufferr then add 10µl phenylamine citrate and 50µl 2,4-dinitrophenylhydrazine, incubate at 37 °C for 20min and record the OD value at 520nm. One unit of AST is the amount of enzyme that will generate 1µmole of pyruvate per minute at pH7.4 at 37 °C.

Calculation

$$\text{AST specific activity} = \frac{OD \times d}{t} / \text{amount of protein}$$

Where:

d - dilution factor

t - reaction time in minutes

The specific activity of recombinant human AST is 4.9 U/mg.

[IDENTIFICATION]

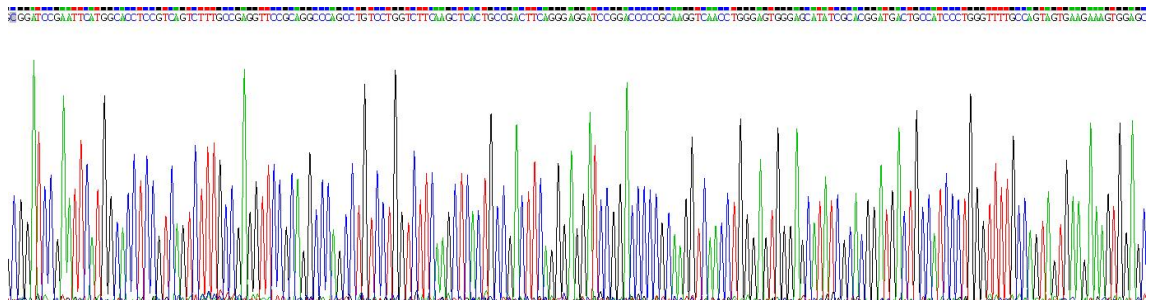


Figure 1. Gene Sequencing (extract)

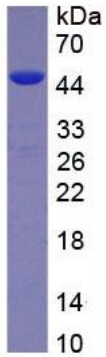


Figure 2. SDS-PAGE

Sample: Active recombinant AST, Human

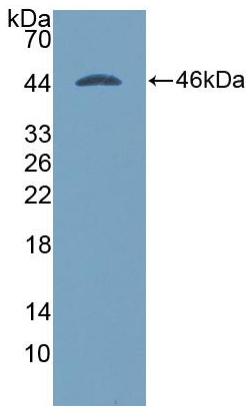


Figure 3. Western Blot

Sample: Recombinant AST, Human;

Antibody: Rabbit Anti- Human AST Ab (PAB214Hu01)

[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.