

APB214Mu01 100µg

**Active Aspartate Aminotransferase (AST)** 

Organism Species: Mus musculus (Mouse)

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: >98%** 

**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

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**Applications:** Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.9

Predicted Molecular Mass: 47.4kDa

Accurate Molecular Mass: 43kDa 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]

MAPPSVFAQVPQAPPVLVFKLTADFRDDPDPRKVNLGVGAYRTDESQPWVLPVVRKVEQKIANDNSLNHEYLPILGLAEFRSCASRLV LGDNSLAIRENRVGGVQSLGGTGALRIGADFLGRWYNGTDNKNTPIYVSSPTWENHNAVFSAAGFKDIRPYCYWDAEKRGLDLQGFLN DLENAPEFSIFVLHACAHNPTGTDPTPEQWKQIAAVMQRRFLFPFFDSAYQGFASGDLEKDAWAIRYFVSEGFELFCAQSFSKNFGLY NERVGNLTVVGKESDSVLRVLSQMEKIVRITWSNPPAQGARIVAATLSDPELFKEWKGNVKTMADRILTMRSELRARLEALKTPGTWS HITEOIGMFSFTGLNPKOVEYLVNEKHIYLLPSGRINMCGLTTKNLDYVATSIHEAVTKIO

#### [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  $\alpha$ -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 10µl different concentation recombinant AST(the blank tube add 10µl phosphate buffer) to 50µl mixture substrate contianing 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

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at 520nm. Standard curve prepare by double dilute  $2\mu M$  pyruvate with phosphate bufferr then add  $10\mu I$  phenylamine citrate and  $50\mu I$  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  $1\mu mole$  of pyruvate per minute at pH7.4 at 37 °C.

Calculation

$$OD \times d$$

AST specific activity= t /amount of protein

Where:

d - dilution factor

t - reaction time in minutes

The specific activity of recombinant rat AST is 6.3U/mg.

#### [ IDENTIFICATION ]

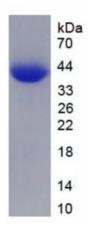


Figure 1. SDS-PAGE

Sample: Active recombinant AST, Mouse

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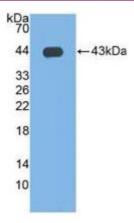


Figure 2. Western Blot

Sample: Recombinant AST, Mouse;

Antibody: Rabbit Anti- Mouse AST Ab (PAB214Mu01)

## [ 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.