APB236Ra01 100µg Active Plasminogen (Plg) Organism Species: Rattus norvegicus (Rat) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Glu191~Arg433

Tags: N-terminal His-tag

Purity: >95%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 7.9

Predicted Molecular Mass: 31.2kDa

Accurate Molecular Mass: 38kDa 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.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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.

[<u>SEQUENCE</u>]

EKYEGKISKT MSGLDCQSWD SQSPHAHGYI PAKFPSKNLK MNYCRNPDGE PRPWCFTTDP NKRWEYCDIP RCTTPPPPG PTYQCLKGRG ENYRGTVSVT ASGKTCQRWS EQTPHRHNRT PENFPCKNLE ENYCRNPDGE TAPWCYTTDS QLRWEYCEIP SCGSSVSPDQ SDSSVLPEQT PVVQECYQGN GKSYRGTSST TNTGKKCQSW VSMTPHSHSK TPANFPDAGL EMNYCRNPDN DQR

[ACTIVITY]

Plasminogen (Plg) can be converted into active plasmin by tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), kallikrein, and factor XII (Hageman factor). Plasmin can dissolve fibrin blood clots, act on many other processes such as embryonic development, tissue remodeling, inflammation and tumor invasion. Plasmint also can activates collagenases, weakens the walls of the Graafian follicle, cleaves fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor. Besides, Actin Beta (ACTb) has been identified as an interactor of Plg, thus a binding ELISA assay was conducted to detect the interaction of

recombinant rat Plg and recombinant rat ACTb. Briefly, Plg were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to ACTb-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-Plg pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of of Plg and ACTb was shown in Figure 1, and this effect was in a dose dependent manner.



Figure 1. The binding activity of Plg with ACTb.



Figure 2. Gene Sequencing (extract)

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	kDa 70
Contract of	44
	33
and the second	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant Plg, Rat



Figure 4. Western Blot

Sample: Recombinant Plg, Rat;

Antibody: Rabbit Anti-Rat Plg Ab (PAB236Ra01)