Coud-Clone Corp.

APB217Hu61 100µg Active Hyaluronidase (HAase) Organism Species: *Homo sapiens* (Human) *Instruction manual* 

#### FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

#### [PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Phe22~Trp435 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: 6.9 Predicted Molecular Mass: 47.6kDa Accurate Molecular Mass: 50kDa as determined by SDS-PAGE reducing conditions.

### [<u>USAGE</u>]

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

# Cloud-Clone Corp.

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>]

FRGPLLPNRPFTTVWNANTQWCLERHGVDVDVSVFDVVANPGQTFRGPDMTIFYSSQLGTYPYYTPTGEPV FGGLPQNASLIAHLARTFQDILAAIPAPDFSGLAVIDWEAWRPRWAFNWDTKDIYRQRSRALVQAQHPDWP APQVEAVAQDQFQGAARAWMAGTLQLGRALRPRGLWGFYGFPDCYNYDFLSPNYTGQCPSGIRAQNDQLGW LWGQSRALYPSIYMPAVLEGTGKSQMYVQHRVAEAFRVAVAAGDPNLPVLPYVQIFYDTTNHFLPLDELEH SLGESAAQGAAGVVLWVSWENTRTKESCQAIKEYMDTTLGPFILNVTSGALLCSQALCSGHGRCVRRTSHP KALLLLNPASFSIQLTPGGGPLSLRGALSLEDQAQMAVEFKCRCYPGWQAPWCERKSMW

### [ACTIVITY]

Hyaluronidase (HAase) is a general term for enzymes that can hydrolyze hyaluronic acid. It is an enzyme that can reduce the activity of hyaluronic acid in the body, thereby improving the fluid permeability in tissues. When used in the human body, it can temporarily reduce the viscosity of the intercellular matrix, promote subcutaneous infusion, locally accumulated exudate or blood to accelerate diffusion and facilitate absorption, and is an important drug dispersant. Clinically used as a drug penetrating agent to promote drug absorption, promote local edema or hematoma dissipation after surgery and trauma. Human HAase consists of 435 amino acids which contains a signal peptide of 1-21 amino acids and it shares 74% and 75% amino acid sequence homology with mouse and rat respectively. The activity assay of recombinant human HAase was measured by its ability to hydrolyze the substrate hyaluronic acid. The rhHAase was diluted to 0.5 ug/ml in 8.46 mM NaH2PO4, 11.54 mM Na2HPO4, 77 mM NaCl, 0.1 mg/ml BSA, pH 7. 50 ul 0.5 ug/ml rhHAase was added into the microplate and start the reaction by adding 50 µL of 0.3 mg/ml substrate which was diluted in 300mM NaH2PO4, pH 5.35. Incubated at 37 ° C for 5min and add 50 ul reaction mixture to 250 ul 1 mg/ml BSA in 24 mM sodium acetic, 79 mM acetic acid, pH 3.75. Incubated at room temperature for 10min and read at a wavelength of 600 nm. The specific activity of recombinant human HAase is >30 pmol/min/µg.

## Cloud-Clone Corp.



	OD600	hyaluronic acid (uM)
	0.3439	0.125
	<mark>0.194</mark> 6	0.0625
8	0.092	0.03125
8	0.0435	0.015625
	0.0166	0.0078125
8	0.0054	0.00390625

Figure 1. The standard curve of hyaluronic acid

One unit of enzyme activity is defined as the 1 µg of enzyme required to convert 1 pmol of hyaluronic acid in 1min.

Specific Activity (pmol/min/µg)=  $\frac{\Delta OD * F}{T * N}$ 

△OD=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of hyaluronic acid)

T=Time

#### [IDENTIFICATION]

kDa 70
44
33
26
22
18
14
10

Figure 2. SDS-PAGE

Sample: Active recombinant HAase, Human

# Cloud-Clone Corp.

### [<u>IMPORTANT NOTE</u>]

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.