#### APF709Hu01 100µg Active Peptidyl Arginine Deiminase Type IV (PADI4) 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: Prokaryotic expression. Host: *E. coli* Residues: Met1~Pro300 Tags: N-terminal His-tag Purity: >95% 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: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.0 Predicted Molecular Mass: 36.3kDa Accurate Molecular Mass: 36kDa 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.

# Cloud-Clone Corp.

**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.

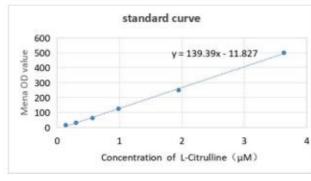
## [ <u>SEQUENCE</u> ]

```
MAQGTLIRVT PEQPTHAVCV LGTLTQLDIC SSAPEDCTSF SINASPGVVV
DIAHGPPAKK KSTGSSTWPL DPGVEVTLTM KVASGSTGDQ KVQISYYGPK
TPPVKALLYL TGVEISLCAD ITRTGKVKPT RAVKDQRTWT WGPCGQGAIL
LVNCDRDNLE SSAMDCEDDE VLDSEDLQDM SLMTLSTKTP KDFFTNHTLV
LHVARSEMDK VRVFQATRGK LSSKCSVVLG PKWPSHYLMV PGGKHNMDFY
VEALAFPDTD FPGLITLTIS LLDTSNLELP EAVVFQDSVV FRVAPWIMTP
```

### [ACTIVITY]

Peptidylarginine deiminase 4 (PADI4) is an enzyme that plays an important role in the organism. It mainly catalyzes the conversion of arginine residues in proteins into citrulline residues. PADI4 plays a key role in various physiological processes such as cell differentiation and immune response. The abnormal expression or change in its activity is closely related to autoimmune diseases like rheumatoid arthritis and systemic lupus erythematosus, making it an important target in the research of these related diseases. The activity of recombinant human PADI4 is determined by its ability to catalyze Benzoyl-arginine ethyl ester (BAEE) to form citrulline. The reaction was performed in 50 mM Tris, 0.1 M NaCl, 10 mM CaCl2, 5 mM DTT solution (Assay Buffer, pH 7.5). A total volume of 300  $\,\mu$  L was achieved by adding 75  $\,\mu$  L of 100  $\,\mu$  g/mL PADI4 to 75  $\,\mu$  L of 10 mM BAEE. After 30-minute incubation at 37  $^\circ$  C, the reaction mixture was acidified with color-developing solution containing ammonium iron sulfate and measured at 540 nm .

## Cloud-Clone Corp.



OD540nm	L-Citrulline ( uM)
3.6322	500
1.9467	250
0.9838	125
0.5679	62.5
0.3021	31.25
0.1386	15.625

M-Figure 1. The standard curve of L-Citrulline

One unit of enzyme activity is defined as the 1 µg of enzyme required to convert 1 pmol of Benzoyl-arginine ethyl ester (BAEE) to citrulline in 1 min at 37°C. The specific activity of recombinant human PADI4 is 5000 pmol/min/µg.

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

△OD=Adjusted for Substrate Blank

F=Conversion Factor(convert from standard curve of p-nitrocatechol)

T= Time

N=Amount of enzyme

### [IDENTIFICATION]

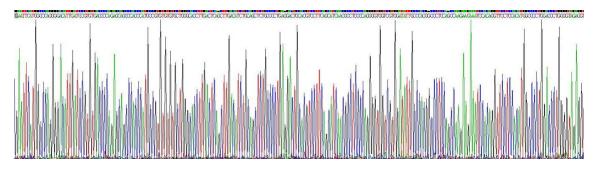


Figure 2. Gene Sequencing (extract)

## Cloud-Clone Corp.

	kDa 70
6 M	44
-	33
	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant PADI4, Human

#### [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.