Coud-Clone Corp.

APA006Hu01 10µg Active Amphiregulin (AREG) 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: Ser20~Asp100

Tags: Two N-terminal Tags, His-tag and GST-tag

**Purity: >92%** 

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 5.1

Predicted Molecular Mass: 38.6kDa

Accurate Molecular Mass: 44kDa 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.

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## [ <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 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]

S GHYAAGLDLN DTYSGKREPF SGDHSADGFE VTSRSEMSSG SEISPVSEMP SSSEPSSGAD YDYSEEYDNE PQIPGYIVDD

## [ACTIVITY]

Amphiregulin (AREG) is a well-characterized member of the epidermal growth factor (EGF) family and is one of the ligands of the EGF receptor (EGFR). AREG plays a key role in mammalian development and in the control of branching morphogenesis in various organs. Furthermore, AREG participates in a wide range of physiological and pathological processes activating the major intracellular signalling cascades governing cell survival, proliferation and motility. Besides, EGF has been identified as an interactor of AREG, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human AREG and recombinant rat EGF. Briefly, AREG was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ l were then transferred to EGF-coated microtiter wells and incubated for 1h at 37°C.

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Wells were washed with PBST and incubated for 1h with anti-AREG pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50  $\mu$ L stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human AREG and recombinant rat EGF was shown in Figure 1, the EC50 for this effect is 0.1 ug/mL.

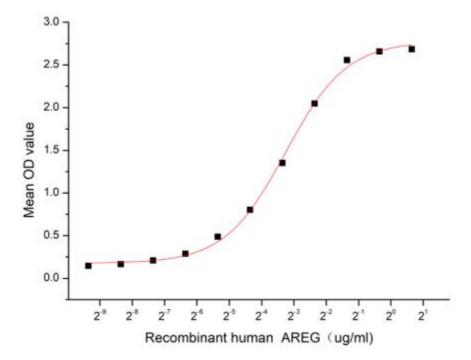


Figure 1. The binding activity of recombinant human AREG and recombinant rat EGF

### [IDENTIFICATION]

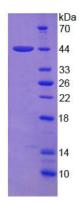


Figure 2. SDS-PAGE

Sample: Active recombinant AREG, 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.