APB112Mu61 100µg Active Complement Component 1, R Subcomponent (C1r) Organism Species: *Mus musculus (Mouse) 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: Met1~Asn707

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: 5.4

Predicted Molecular Mass: 81.7kDa

Accurate Molecular Mass: 33&18kDa 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.

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

MWLFALLVTLFYGVEGSIYLPQKLYGEVTSPLYPKPYPSDLETTTVITVPMGYRVKLVFWQFDVEPS EGCFYDYVKISADKQTLGRFCGQLDSPLGNPPGSKEFMSQGNKMLLTFHTDFSNEENGTIMFYKGFL AYYQAVDLDECASQPNSVEEGLQPRCQHLCHNYVGGYFCSCHPGYELQKDGQSCQAECSSELYTEPS GYVSSLEYPQPYPPDLRCNYSIRVERGLTVHLKFLDPFEIDDHQQVHCPYDQLQIYANGKNLGEFCG KQRPPDLDTSSNAVDLLFFTDESGDSRGWKLHYTTETIKCPQPKALDEFTIIQDPQPQYQFRDYFIV TCKQGYQLMEGNQALLSFTAVCQNDGTWHRAMPRCKIKNCGQPQSLSNGDFRYITTKGVTTYEASIQ YHCHEPYYKMLTRAGSSESMRGIYTCTAQGIWKNEEEGEKMPRCLPVCGKPVNPVTQKERIIRGQPA RPGNFFWQAFTTTHGRGGGALLGDRWILTAAHTIYPKHHNKENDNANPKMLVFLGHTNVEQIKKLGH HPVRRVIIHPDYRQDEPNNFEGDIALLELENSVTLGPELLPICLPDNETFYGQGLMGYVSGFGITED KLAFDLRFVRLPVADSEACQRWLQTKKDTSPFSQNMFCSGDPAVQQDACQGDSGGVFAVRDRNRDIW VATGIVSWGIGCGEGYGFYTKVLNYVDWIKKEMGDEN

## [ACTIVITY]

The classical complement pathway plays a major role in innate immunity against infection. This pathway is triggered by C1, a multimolecular complex composed of the recognition protein C1q and two serine proteases, C1r and C1s. Following the C1q recognition, C1r is autoactivated, and in turn activates C1s, which cleaves C4 and C2, the C1 substrates. Both C1r and C1s activation involve cleavage of a specific Arg-lle bond, converting single-chain proenzymes into active proteases of disulfide bond-linked chains (A and B). The full-length (amino acid residues 1-707)

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of mouse C1r was expressed which activity was measured by its ability to cleaves a thioester substrate Z-Lys-SBzI+HCI. The reaction was performed in 50 mM Tris, pH 7.5 (Assay Buffer), initiated by addition 50  $\mu$  L of various concentrations of C1r (diluted by Assay Buffer) to 50  $\mu$ I substrate mixture of 0.2mM Z-Lys-SBzI+HCI and 0.2 mM DTNB. The final well serves as a negative control with no C1r, replaced with 50  $\mu$ I assay buffer. Then read in kinetic mode for 5 minutes at an absorbance of 405 nm. The specific activity of recombinant mouse C1r is > 500 pmol/min/ $\mu$ g.

Specific Activity (pmol/min/ug)=

Adjusted V<sub>max</sub>\* (OD/min) x well volume (L) x 1012 pmol/mol

ext. coeff\*\* (M-1cm-1) x path corr.\*\*\* (cm) x amount of enzyme (ug)

\*Adjusted for Substrate Blank \*\*Using the extinction coefficient 13260 M<sup>-1</sup>cm<sup>-1</sup> \*\*\*\*Using the path correction 0.320 cm

#### [IDENTIFICATION]

kDa 70
44
33
26
22
18
14
10

Figure 1. SDS-PAGE

Sample: Active recombinant C1r, Mouse

#### [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.