### Recombinant Human Complement Component C1r

**Catalog Number:** 1807-SE

**Source**  
Mouse myeloma cell line, NS0-derived  
Met1-Asp705, with a C-terminally His tag  
Accession # NP_001724

**N-terminal Sequence Analysis**  
Ser18 (A chain) & Ile464 (B chain)

**Structure / Form**  
Disulfide-linked heterodimer

**Predicted Molecular Mass**  
51 kDa (A chain) & 28 kDa (B chain)

**SPECIFICATIONS**

<table>
<thead>
<tr>
<th>SDS-PAGE</th>
<th>Activity</th>
<th>Endotoxin Level</th>
<th>Purity</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 kDa &amp; 40 kDa, reducing conditions</td>
<td>Measured by its ability to cleave a colorimetric peptide substrate, N-carbobenzyloxy-Gly-Arg-ThioBenzyI ester (Z-GR-SBzl), in the presence of 5,5′Dithio-bis (2-nitrobenzoic acid) (DTNB). Edwards, K.M. et al. (1999) J. Biol. Chem. 274:30468. The specific activity is &gt;1,500 pmol/min/µg, as measured under the described conditions.</td>
<td>&lt;1.0 EU per 1 µg of the protein by the LAL method.</td>
<td>&gt;95%, by SDS-PAGE under reducing conditions and visualized by silver stain.</td>
<td>Lyophilized from a 0.2 µL solution in Tris and NaCl. See Certificate of Analysis for details.</td>
</tr>
</tbody>
</table>

**REFERENCE**


**Activity Assay Protocol**

**Materials**

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Human Complement Component C1r (rhC1r) (Catalog # 1807-SE)
- Substrate: Z-Gly-Arg-SBzl (MP Biomedicals, Catalog # SB007), 10 mM stock in DMSO
- 5,5′Dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130), 10 mM stock in DMSO
- 96 well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute rhC1r to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer with 200 µM of DTNB.
3. Load 50 µL of the diluted rhC1r into a clear plate, and start the reaction by adding 50 µL of the Substrate/DTNB mixture to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate Mixture without any rhC1r.
4. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.
5. Calculate specific activity:

   \[
   \text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\max}}{\text{ext. coeff}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol ext. coeff}^* \text{ (M}^{-1}\text{cm}^{-1}) \times \text{path corr.*** (cm)} \times \text{amount of enzyme (µg)}}
   \]

**Final Assay Conditions**

- rhC1r: 0.100 µg
- DTNB: 100 µM
- Substrate: 100 µM

**PREPARATION AND STORAGE**

**Reconstitution**  
Reconstitute at 100 µg/mL in sterile 50 mM Tris and 150 mM NaCl, pH 7.5.

**Shipping**  
The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

**BACKGROUND**

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 (1). Both C1r and C1s activation involve cleavage of a specific Arg-ile bond, converting single-chain proenzymes into active proteases of disulfide bond-linked chains (A and B) (2). The A chains contain multiple domains in the order of CUB1-EGF-CUB2-CCP1-CCP2-Activation Peptide. The B chains contain the serine protease catalytic domain. The full-length (amino acid residues 1-705) of human C1r was expressed, which had the Leu152 natural variant (3). The purified protein corresponded to the processed active form, with A and B chains starting at residue Ser18 and Ile464, respectively.

**References:**


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