DESCRIPTION
Source  Mouse myeloma cell line, NS0-derived  
A1a21-Leu752, with a C-terminal 6-His tag  
Accession # P06681  

N-terminal Sequence  A1a21  
Analysis  

Structure / Form  Pro form  
Predicted Molecular Mass  82 kDa  

SPECIFICATIONS
SDS-PAGE  103 kDa, reducing conditions  
The specific activity is >100 pmol/min/µg, as measured under the described conditions.  
Endotoxin Level  <1.0 EU per 1 µg of the protein by the LAL method.  
Purity  >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.  
Formulation  Supplied as a 0.2 µm filtered solution in Tris, NaCl and CaCl₂. See Certificate of Analysis for details.  

Activity Assay Protocol  
Materials  
- Assay Buffer: 50 mM Tris, pH 8.0  
- Recombinant Human Complement Component C2 (rhC2) (Catalog # 1936-SE)  
- Substrate: Z-Gly-Arg-SBZL (MP Biomedicals, Catalog # SB007)  
- 5,5'Dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130)  
- 96 well Clear Plate (Costar, Catalog # 92592)  
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent  

Assay  
1. Dilute rhC2 to 4 µg/mL in Assay Buffer.  
2. Dilute Substrate to 200 µM in Assay Buffer with 200 µM of DTNB.  
3. Load 50 µL of the diluted rhC2 into a plate, and start the reaction by adding 50 µL of the Substrate/DTNB mixture to wells. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of the Substrate mixture.  
4. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.  
5. Calculate specific activity:  
\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times (\text{OD/min} \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol ext. coeff} \times \text{path corr.} \times \text{amount of enzyme (µg)})}{100}
\]

*Adjusted for Substrate Blank  
**Using the extinction coefficient 13260 M⁻¹cm⁻¹  
***Using the path correction 0.32 cm  
Note: the output of many spectrophotometers is in mOD  

Final Assay Conditions  Per Well:  
- rhC2: 0.2 µg  
- DTNB: 100 µM  
- Substrate: 100 µM  

PREPARATION AND STORAGE  
Shipping  The product is shipped with polar packs. 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 opening.  

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 (1). After activation by C1, the single-chain form of C2 (amino acid residues 21-752) becomes two chains, which are referred to as C2A and C2B. C2A (residues 244-752) consists of a vWF domain (residues 254-452) and a serine protease domain (residues 466-752). C2B (residues 21-243) contains 3 Sushi (SCR) domains. C2A, then combines with complement factor 4B to generate the C3 or C5 convertase. The full length of human C2 was expressed, and the purified protein corresponded to the single-chain form with the peptidase activity as described in the Activity Assay Protocol.  

References:  