Recombinant Mouse Complement Component C2

Catalog Number: 6725-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse Complement Component C2 protein Met1-Leu760, with a C-terminal 6-His tag. Accession # O70350
N-terminal Sequence Analysis	Leu20 & Lys251
Predicted Molecular Mass	58 kDa and 84 kDa

SPECIFICATIONS

SDS-PAGE	66-75 kDa & 90-110 kDa, reducing conditions
Activity	Measured by its ability to cleave a colorimetric peptide substrate, N-carbobenzyloxy-Gly-Arg-ThioBenzyl 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 >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 Sodium Acetate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 8.0
- Recombinant Mouse Complement Component C2 (rmC2) (Catalog # 6725-SE)
- Substrate: Z-GR-SBzl, 10 mM stock in DMSO
- 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 10 mM stock in DMSO
- Clear 96-well Plate
- Plate Reader

Assay

1. Dilute rmC2 to 4 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM with 200 µM DTNB in Assay Buffer.
3. Load into a plate 50 µL of 4 ng/µL rmC2, and start the reaction by adding 50 µL of 200 µM Substrate. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
4. Read plate at a wavelength of 405 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.320 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rmC2: 0.200 µg
- Substrate: 100 µM
- DTNB: 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

Complement Component C2 (C2) is a serine protease that is part of the classical pathway of the complement system. 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 proteolytic activation by C1, the single chain form of C2 (amino acid residues 19-760) becomes two chains, which are referred to as C2A and C2B. C2A (residues 251-760) consists of a vWF domain (residues 260-457) and a serine protease domain (residues 480-760). After the activation by C1, C2A combines with complement factor 4B to generate the C3 or C5 convertase. The full length mouse C2 was expressed, and the purified protein consists of a mixture of single chain and C2A form based on the N-terminal sequencing data.

References:

1. Arlaud, G.J. *et al.* (2002) Biochem. Soc. Trans. **30**:1001.

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