Recombinant Human Complement Component C1rLP HA-tag His-tag

Catalog Number: 11614-CP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Complement Component C1rLP protein Cys36-Asp487 with N-terminal HA (YPYDVPDYA) and 6-His tags Accession # NP_057630.1
N-terminal Sequence Analysis	Tyr
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	70-76 kDa, under 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 >30 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Human Complement Component C1rLP (rhC1rLP) (Catalog # 11614-CP)
- Substrate: Z-GR-SBzl,10 mM stock in DMSO
- 5,5'Dithio-bis (2-nitrobenzoic acid) (DTNB), 10 mM stock in DMSO
- Clear 96-well plate
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute rhC1rLP to 40 ng/µL in Assay Buffer.
2. Prepare a Substrate Mixture containing 400 µM of Z-GR-SBzl and 200 µM of DTNB in Assay Buffer.
3. In a plate, load 50 µL of 40 ng/µL rhC1rLP and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at 405 nm (absorbance) in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

***Using the path correction of 0.320 cm

Final Assay Conditions

Per Well:

- rhC1rLP: 2 µg
- Z-GR-SBzl: 200 µM
- DTNB: 100 µM

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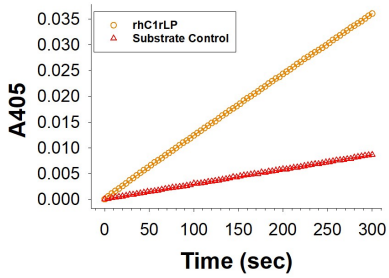
PREPARATION AND STORAGE

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.

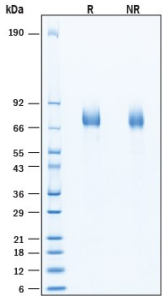
DATA

Enzyme Activity



Recombinant Human Complement Component C1rLP HA-tag His-tag Protein Enzyme Activity. Recombinant Human Complement Component C1rLP HA-tag His-tag (Catalog # 11614-CP) is 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).

SDS-PAGE



Recombinant Human Complement Component C1rLP HA-tag His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human Complement Component C1rLP HA-tag His-tag Protein (Catalog # 11614-CP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 70-76 kDa, under reducing conditions.

BACKGROUND

Recombinant human Complement C1r subcomponent-like protein (C1rLP), also known as C1r-like serine protease analog protein (CLSPa) is a 487 residue glycosylated, secreted serine peptidase-like protein (1, 2). It contains an N-terminal signal peptide, a CUB domain for substrate and protein-protein interaction, a truncated complement control protein module, and a trypsin-like serine protease domain with a characteristic catalytic triad but lacks an activation consensus peptide and an EGF domain found in homologous complement cascade proteases like C1r protein (1, 2). It is broadly expressed and secreted in tissues and abundantly found in liver, kidney, and myeloid cells where significant expression of serine proteases typically occurs (1). C1rLP has esterolytic activity on peptide thioesters with arginine at the P1 position but lower catalytic efficiency compared to C1r (2). It was reported to mediate cleavage of haptoglobin (3) and subsequently proposed to be of use in hemoglobin-binding therapeutics (4). Complement cascade serine proteases such as C1r play a role in innate immunity but the role of C1rLP in complement-mediated function is unclear as both activation and inhibition roles have been described in the literature (1, 2). C1rLP has been shown to be a pathological marker in immune microenvironments including within acute myocardial infarction and COVID19 as well as several types of cancer such as colon, leukemia, brain, endometrial, and melanoma (5-12). As a pathological marker, it has also been proposed as a potential therapeutic target for the treatment of these diseases (12).

References:

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