Recombinant *S. pyogenes* IdeS His-tag

Catalog Number: 11341-ID

DESCRIPTION

Source	*E. coli*-derived *s. pyogenes* IdeS protein Asp30-Asn339, with an N-terminal Met and C-terminal 6-His tag Accession # YP_006932748.1
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	32-35 kDa, under reducing conditions
Activity	Measured by its ability to cleave human IgG. The DC_{50} is <25 ng, as measured under the described conditions. The DC_{50} is defined as the amount of enzyme required to cleave 50% of 1 µg human IgG in 30 minutes at 37 °C. Use of Recombinant *S. pyogenes* IdeS in the cleavage of other IgGs may require alternative conditions for optimal performance.
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: 25 mM Sodium Phosphate, 150 mM NaCl, pH 7.5
- Recombinant *S. pyogenes* IdeS (rS.p Ides) (Catalog # 11341-ID)
- Purified Human IgG (Catalog # 1-001-A)
- 15% SDS-PAGE gel
- Reducing Sample Buffer
- Gel Staining Reagent

Assay

1. Prepare a curve of rS.p Ides by diluting rS.p Ides to 200, 50, 12.5, 3.125, 0.781, 0.195, and 0.049 µg/mL in Assay Buffer.
2. Dilute Human IgG to 200 µg/mL in Assay Buffer.
3. Combine 20 µL of each rS.p Ides curve dilution and 20 µL of 200 µg/mL Human IgG.
4. Include a control by combining 20 µL of 200 µg/mL Human IgG and 20 µL Assay Buffer.
5. Incubate reaction mixtures and control at 37 °C for 30 minutes.
6. Combine each 10 µL reaction mixture (including controls) with 10 µL of Reducing Sample Buffer. Heat at 95 °C for 3 minutes.
7. Load entire volume of each reaction mixture (20 µL) per lane onto a 15% SDS-PAGE gel and perform electrophoresis.
8. Stain gel and analyze the percent digestion of Human IgG using densitometry for each rS.p Ides curve dilution.
9. Determine the DC_{50} by plotting % digestion vs rS.p Ides concentration (ng) using 4-PL fitting.

Final Assay Conditions

Per Lane:

- rS.p Ides: 1000, 250, 62.5, 15.6, 3.9, 0.98, 0.24, and 0 ng
- Human IgG: 1 µg

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

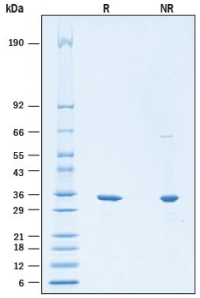
Stability & Storage

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

SDS-PAGE



Recombinant *S. pyogenes* IdeS His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant *S. pyogenes* IdeS His-tag Protein (Catalog # 11341-ID) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 32-35 kDa.

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BACKGROUND

IdeS from Streptococcus pyogenes, originally known as Mac-1, is a unique cysteine protease secreted in mature active form with a high degree of specificity for IgG. The structure of IdeS resembles features of the papain cysteine protease family with two distinct domains that interact through a polar interface containing an active site catalytic triad (1). However, IdeS is unique in that it does not contain disulfide bonds or a propeptide and has a RGD motif, known to be important for recognition by integrins (1). Studies have also found IdeS can inhibit neutrophil effector production of reactive oxygen species to repress immune responses in addition to performing IgG hydrolysis (2). IdeS digests all subclasses of human, and some classes of monkey, rabbit and sheep IgG with limited activity on mouse IgG2a and IgG3. IdeS digests IgG at a specific defined site between two glycines in the lower hinger region of the IgG heavy chain generating a homogenous pool of F(ab')2 and Fc/2 fragments. The lower hinge region is implicated as being important for Fc receptor recognition and complement binding (2-4). The high specificity and generation of homogeneous products has led to development of analytical strategies utilizing IdeS to characterize monoclonal therapeutic antibodies and related products such as antibody-drug conjugates, Fc-fusion proteins and bispecific antibodies (5). Additionally, animal models have provided significant proof of concept for use of IdeS as a therapeutic in treatment of IgG-mediated autoimmune diseases leading to some success using IdeS in clinical trials (2, 6).

References:

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