# Recombinant Streptococcus equi subsp. zooepidemicus IgG endopeptidase IdeZ His-tag

Catalog Number: 11572-IZ

## DESCRIPTION

| | |
|---|---|
| **Source** | *E. coli*-derived IdeZ protein<br>Asp35-Ser349, with an N-terminal Met and C-terminal 6-His tag<br>Accession # WP_014622780.1 |
| **N-terminal Sequence Analysis** | Met |
| **Predicted Molecular Mass** | 36 kDa |

## SPECIFICATIONS

| | |
|---|---|
| **SDS-PAGE** | 35-39 kDa, under reducing conditions |
| **Activity** | Measured by its ability to cleave Human IgG.<br>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 *Streptococcus equi subsp. zooepidemicus* IdeZ in the cleavage of other IgGs may required 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. equi subsp. zooepidemicus* IgG *endopeptidase* IdeZ (rS.e IdeZ) (Catalog # 11572-IZ)
- Purified Human IgG (Catalog # 1-001-A)
- 15% SDS-PAGE gel
- Reducing Sample Buffer
- Gel Staining Reagent

**Assay**

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

**Final Assay Conditions**

Per Lane:

- rS.e IdeZ: 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 dry ice or equivalent. 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

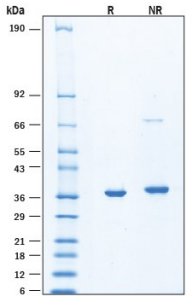


Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

# Recombinant Streptococcus equi subsp. zooepidemicus IgG endopeptidase IdeZ His-tag

Catalog Number: 11572-IZ

**SDS-PAGE**




**Recombinant *Streptococcus equi subsp. zooepidemicus* IgG endopeptidase IdeZ His-tag Protein SDS-PAGE.** 2 µg/lane of Recombinant *Streptococcus equi subsp. zooepidemicus* IgG endopeptidase IdeZ His-tag Protein (Catalog # 11572-IZ) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 35-39 kDa, under reducing conditions.

## BACKGROUND

IdeZ from bacterial pathogen *S. equi subspecies zooepidemicus*, a group C streptococci common in animal infection, is a cysteine protease IgG endopeptidase that acts to ablate immune effector function during infection. As a homolog of the better-known IdeS/MAC from S. pyogenes, IdeZ also contains conserved critical residues that allow it to recognize and cleave at a single site below the hinge of IgG to produce F(ab')2 and Fc fragments with high specificity (1,2). However, IdeZ contains only a single cysteine and can cleave IgG more efficiently under non-reducing conditions (2). The bacterial pathogen is known to have a broad host range and since it is capable of cleaving IgG from multiple species it can cause pathogenesis in many animals such as strangles in horses (1), hemorrhagic pneumonia in dogs in kennels (3), and mastitis in ruminates on farms (4). *S. zooepidemicus* human infection has been reported from direct exposure to infected animals or contracted from infected animal products resulting in nephritis, hospitalization and death as IdeZ is capable of efficient cleavage of all human IgGs (5-8). IdeZ has been reported as a tool in analytical strategies to characterize therapeutic antibodies (9,10) and to cleave proteins post-translationally modified with O-GlcNAc to enable immunoprecipitation analysis (11). IdeZ's broader specificity compared to IdeS means it can more effectively be used in animal model studies, for example due to its ability to more effectively cleave mouse IgG2a and IgG3. IdeZ has also been proposed as a target to develop vaccines to protect animals against group C streptococci (12). Finally, IdeZ has been used in the development of recombinant adeno-associated virus (AAV) vectors for the genetic therapy of rare diseases to eliminate already existing circulating neutralizing antibodies against recombinant AAVs (13).

**References:**

1. Boksha, I.S. *et. al.* (2023) Biochemistry **88**:731.
2. Lannergard, J. and B. Guss (2006) FEMS Microbiol. Lett. **262**:230.
3. Priestnall, S. and K. Erles (2011) Vet. J. **188**:142.
4. Pisoni, G. *et. al.* (2009) J. Dairy Sci.**92**:943.
5. Bradley, S.F. *et. al.* (1991) Rev. Infect. Diseases **13**:270.
6. Balter, S. *et. al.* (2000) Lancet. **355**:1776.
7. Abbott, Y. *et. al.* (2010) J. Med. Microbiol. **59**:120.
8. Pelkonen, S. *et. al.* (2013) Emerg. Infect. Dis. **19**:1041.
9. Bobaly, B. *et. al.* (2017) J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.**1060**:325.
10. Ruppen, I. *et. al.* (2023) J. Pharm. Biomed. Anal. **236**:115743.
11. Machacek, M. *et. al.* (2020) Anal. Biochem. **611**:114001.
12. Velineni, S. and J.F. Timoney (2013) Vaccine. **31**:4129.
13. Elmore, Z.C. *et. al.* (2020) JCI Insight **5**:e139881.



Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475