**Cleavage Sites:**
- human IgG1, IgG3, IgG4: CPAPELLG\*GPSVF
- human IgG2: CPAPPVAGPSVF
- murine IgG2a: CPAAPPLG\*GPSVF
- murine IgG3: CPPGLILG\*GPSVF

**Source:** Cloned from *Streptococcus equi* subspecies *zooepidemicus* and expressed in *E. coli.*

Supplied in: 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 7.5 @ 25°C

**Reagents Supplied with Enzyme:**
- 10X GlycoBuffer 2

**Unit Definition:** One unit is defined as the amount of enzyme required to cleave > 95% of 1 µg of human IgG, in 15 minutes at 37°C in a total reaction volume of 10 µl.

**Unit Definition Assay:** Two fold dilutions of IdeZ Protease (IgG-specific) are incubated with 1 µg of human IgG and 1X GlycoBuffer 2 in a 10 µl reaction. The reaction mix is incubated for 15 minutes at 37°C. Separation of reaction products are visualized by SDS-PAGE.

**Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation.**

**Protease Assay:** After incubation of 800 units of IdeZ Protease (IgG-specific) with 24 µg of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no non-specific proteolytic activity could be detected by SDS-PAGE with Coomassie Blue Detection.

**Heat Inactivation:** 65°C for 10 minutes.
Reaction Protocol:
The quantity of enzyme recommended is sufficient for the deglycosylation of 50 μg of IgG under non-denaturing conditions. Reactions may be scaled-up linearly to accommodate larger amounts of IgG. Optimal incubation times may vary for a particular substrate.

Typical Reaction Conditions are as Follows:
1. Combine up to 50 μg of human IgG and H2O in a total reaction volume of 25 μl.
2. Add 2.5 μl of GlycoBuffer 2 (10X).
3. Add 1 μl of IdeZ Protease (IgG-specific).
4. Incubate at 37°C for 30 minutes.*
5. Prepare IgG sample for SDS-PAGE or mass spectrometry analysis.

*Note: Extend incubation time to 2 hours for murine IgG2a.

Storage: Store at –20°C for up to two years. Avoid multiple freeze/thaw cycles.

Notes:
IdeZ Protease efficiently cleaves human, humanized, chimeric, sheep, rabbit and monkey IgG as well as mouse IgG2a and IgG3. IdeZ Protease will also cleave Fc-fusion proteins, such as Enbrel. IdeZ Protease does not cleave mouse IgG1 or IgG2b, rat, porcine, bovine or goat IgG. It also does not cleave non-IgG isotypes including IgA, IgM, IgD and IgE.

Companion products
Rapid PNGase F Antibody Standard (P6043)
Rapid PNGase F (P0710)
Remove-iT® Endo S (P0741)
Trypsin-ultra™, Mass Spectrometry Grade (P8101)