

## Certificate of Analysis

### IdeZ Protease, Lyophilized

Cat.# V8341 Includes:

Part#	Name	Size
V834A	IdeZ Protease	5,000 units

Cat.# V8345 Includes:

Part#	Name	Size
V834A	IdeZ Protease	25,000 units (5 × 5,000 units)

**Description:** IdeZ (Immunoglobulin-degrading enzyme from *Streptococcus equi* subspecies *zooepidemicus*) is an engineered recombinant protease overexpressed in *Escherichia coli*. IdeZ Protease specifically cleaves IgG molecules below the hinge region to yield F(ab)<sub>2</sub> and Fc fragments.

**Biological Source:** Recombinant strain of *E. coli*.

**Molecular Weight:** IdeZ Protease has a molecular weight of approximately 38kDa.

**Form:** Lyophilized from 50mM sodium phosphate, 150mM NaCl (pH 6.6).

**Storage Conditions:** Store product below -10°C. Prior to use, reconstitute the lyophilized IdeZ Protease with water. Reconstituted IdeZ Protease may be stored at 2-10°C for up to 60 days.

**Expiration Date:** See product label for expiration date.

**Unit Definition:** One unit will cleave ≥95% of 1µg of recombinant monoclonal IgG in 30 minutes at 37°C.

Part# 9PIV834

Revised 2/17



AF9PIV834 0217V834



**Promega**

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### Quality Control Assays

This lot passes the following Quality Control specifications:

**Purity:** ≥95% as determined by SDS-PAGE analysis.

**Activity:** Unit activity for each lot of IdeZ Protease is confirmed by the cleavage of recombinant monoclonal IgG as analyzed by SDS-PAGE.

#### PRODUCT USE LIMITATIONS, WARRANTY DISCLAIMER

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Signed by:

R. Wheeler, Quality Assurance

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## Protocol for Antibody Fragmentation

### Reconstitution

1. Add 100µl of deionized water to one tube of the lyophilized IdeZ Protease to make a 50 unit/µl solution.

### Antibody Fragmentation

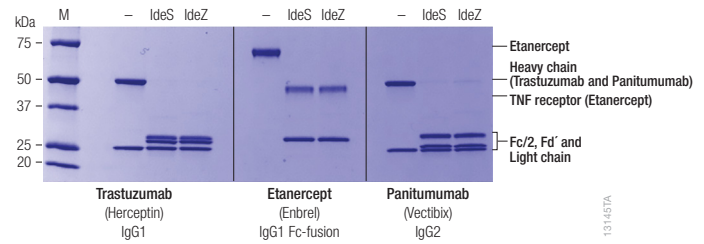
1. Add the desired amount of IgG (up to 5mg) in digestion buffer or other compatible buffer<sup>1</sup>.
2. Add IdeZ Protease to the IgG sample:
  - Add 1 unit of IdeZ Protease per 1µg of IgG to be digested.
  - For example, add 1µl (50 units) of reconstituted IdeZ Protease to digest 50µg of IgG.
3. Incubate sample at 37°C for 30–60 minutes<sup>2</sup>.

<sup>1</sup>IdeZ Protease is most active in buffers at or near neutral pH. The recommended digestion buffer is 50mM sodium phosphate, 150mM NaCl (pH 6.6), but many common biological buffers such as Tris or PBS also can be used. Buffers outside this pH range (e.g., acetate buffers) may be used, but the incubation time and/or enzyme amount may have to be optimized on a case-by-case basis.

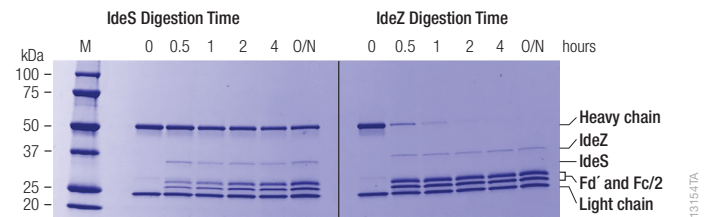
<sup>2</sup>Mouse IgG2a and IgG3 typically require 2–4 hours for complete digestion. In some cases, human IgG2 may require incubation beyond 60 minutes.

### Notes

- IgG concentration ideally should be in the range of 1–20mg/ml.
- IdeZ Protease efficiently cleaves human, humanized, chimeric, sheep, rabbit and monkey IgGs as well as mouse IgG2a and IgG3. IdeZ Protease also will cleave many Fc-fusion proteins as well as antibody-drug conjugates (ADCs; Figures 1 and 2).
- IdeZ Protease does not cleave mouse IgG1/IgG2b, rat, porcine, bovine or goat IgG. IdeZ Protease also does not cleave non-IgG isotypes including IgA, IgM, IgD and IgE.
- IdeZ Protease cleaves at a single site below the hinge at the same location as IdeS Protease (Cat.# V7511, V7515).
- The IdeZ Protease has a histidine tag for easy removal.
- For downstream analysis of fragments by LC-MS, best results are obtained following reduction and denaturing of the IdeZ-digested fragments.
- Purified F(ab)<sub>2</sub> fragments can be obtained after digestion by incubating the digest for 30–60 minutes with Magne™ Protein A Beads (Cat.# G8781).
- IdeZ Protease can be used in the same reaction as PNGase F (Cat.# V4831) to perform fragmentation and removal of Fc glycans in a single step using the recommended digest buffer. Ten units of PNGase F may be sufficient to remove Fc glycans from 50µg of IgG in 2 hours at 37°C, although some optimization of PNGase F amount and incubation time may be required.



**Figure 1. Digestion of panel of therapeutic IgGs and Fc-fusion proteins.** Therapeutic IgG or fusion protein (50µg) was digested with 50 units of IdeS Protease or IdeZ Protease for 30 minutes at 37°C in a final volume of 25µl in the recommended digestion buffer (50mM sodium phosphate, 150mM NaCl [pH 6.6]). Undigested controls and samples were analyzed by SDS-PAGE under reducing conditions.



**Figure 2. Digestion of recombinant mouse IgG2a with IdeS Protease and IdeZ Protease.** Recombinant mIgG2a anti-hCD20 (50µg; Invivogen) was digested with 50 units of IdeS Protease or IdeZ Protease for various time points at 37°C in a final volume of 25µl in the recommended digestion buffer (50mM sodium phosphate, 150mM NaCl [pH 6.6]). Samples were analyzed by SDS-PAGE under reducing conditions. IgG2a is completely digested in 2 hours with IdeZ Protease but is only partially digested with IdeS Protease even after an overnight (O/N) digestion.

## Related Products

Product	Size	Cat.#
IdeZ Protease, Frozen	2,000 units	V8342
IdeS Protease (lyophilized)	5,000 units	V7511
	25,000 units (5 × 5,000 units)	V7515
IdeS Protease, Frozen	2,000 units	V7512
PNGase F	500 units	V4831
Magne™ Protein A Beads, 20% Slurry	1ml	G8781
ISOQUANT® Isoaspartate Detection Kit*	100 assays	MA1010
Protein Deglycosylation Mix	20 reactions	V4931
Trypsin/Lys-C Mix, Mass Spec Grade	100µg	V5072
Trypsin Gold, Mass Spectrometry Grade	100µg	V5280
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)	V5111
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)	V5113
rLys-C, Mass Spec Grade	15µg	V1671
Asp-N, Sequencing Grade	2µg	V1621
Glu-C, Sequencing Grade	50µg (5 × 10µg)	V1651

\*Not For Medical Diagnostic Use.