Certificate of Analysis

IdeS Protease

Part# 9PIV751

Revised 2/17

Cat.#	Name	Size
V7511	IdeS Protease	5,000 units
V7515	IdeS Protease	5 × 5,000 units

Description: IdeS (Immunoglobulin-degrading enzyme from *Streptococcus pyogenes*) is an engineered recombinant protease overexpressed in *Escherichia coli*. IdeS Protease specifically cleaves IgG molecules below the hinge region to yield $F(ab')_2$ and Fc fragments.

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

Molecular Weight: IdeS Protease has a molecular weight of approximately 37kDa.

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

Storage Conditions: Store product at –30°C to –10°C. Prior to use, reconstitute the lyophilized IdeS Protease with water. Reconstituted IdeS 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.

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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 IdeS is confirmed by the cleavage of recombinant monoclonal IgG as analyzed by SDS-PAGE.

Signed by:

R. Wheeler, Quality Assurance

Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

Effective June 29, 2016, a settlement between Promega Corporation and Genovis AB maintains full freedom for continued offering of Promega's IdeS and IdeZ Proteases for markets worldwide.

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Printed in USA. Revised 2/17.

Usage Information

1. Antibody Fragmentation

A. Reconstitution

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

B. Protocol

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

*IdeS 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.

Notes

- IgG concentration ideally should be in the range of 0.5–20mg/ml.
- IdeS Protease efficiently cleaves human, humanized, chimeric, sheep, rabbit and monkey IgGs and has moderate activity against mouse IgG2a and IgG3. IdeS will also cleave many Fc-fusion proteins as well as antibody drug conjugates (ADCs).
- Cleavage of IgGs can be readily determined by SDS-PAGE (Figure 1).
- IdeS Protease does not cleave mouse IgG1/IgG2b, rat, porcine, bovine or goat IgG. Also, it does not cleave non-IgG isotypes including IgA, IgM, IgD and IgE.
- For cleavage of mouse IgG2a and mouse IgG3, increase the amount of IdeS Protease (5-fold to 10-fold is suggested as a starting point) and the incubation time (2 hours to overnight).
- The IdeS 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 IdeS-digested fragments.
- Purified F(ab')$_2$ fragments can be obtained after digestion by incubating the digest for 30–60 minutes with Magne™ Protein A Beads (Cat.# G8781).
- IdeS 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.

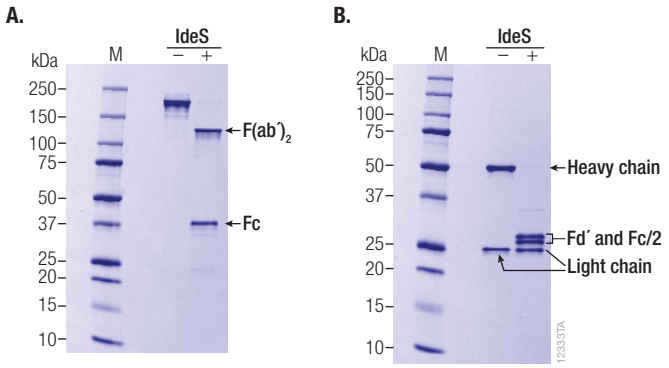

Figure 1. Results of trastuzumab digestion separated under nonreducing and reducing conditions. Trastuzumab (50µg) was incubated with 50 units of IdeS Protease for 30 minutes at 37°C using the recommended digestion buffer (50mM sodium phosphate, 150mM NaCl [pH 6.6]). The digestion products were analyzed by SDS-PAGE under nonreducing (**Panel A**) and reducing (**Panel B**) conditions.

2. Related Products

Product	Size	Cat.#
PNGase F	500 units	V4831
Magne™ Protein A Beads, 20% Slurry	1ml	G8781
ISOQUANT® Isoaspartate Detection Kit*	100 assays	MA1010
ADCC Reporter Bioassay, Core Kit	1 each	G7010
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

*Not For Medical Diagnostic Use.

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