

## Product components

| Components               | Component number | Concentration | Size (1,000 U) |
|--------------------------|------------------|---------------|----------------|
| IdeZ Protease            | RM20583          | 10,000 U/mL   | 100 µL         |
| 10X IdeZ Reaction Buffer | RM20872          | 10X           | 1 mL           |

## Product Description

IdeZ Protease (IgG-specific) is a recombinant antibody-specific protease that recognizes IgG of specific species and subtypes, such as human and mouse IgG. It cleaves specifically at a single recognition site below the hinge region, producing uniform F(ab')<sub>2</sub> and Fc fragment pools.

## Source

Cloned from *Streptococcus equi* subsp. *zooepidemicus* and expressed in *E. coli*.

## Definition of Activity Unit

One unit (U) is defined as the amount of enzyme required to cleave >95% of 1 µg of human IgG in a 10 µL reaction within 15 min at 37 °C.

## Storage Buffer

1X PBS without K, pH 7.4 @ 25 °C, 50% Glycerol

## Store

-20 °C

## Reaction Conditions

10X IdeZ Reaction Buffer, at 37 °C

## Composition of 1X IdeZ Reaction Buffer

1X PBS without K, pH 7.4 @ 25 °C

## Thermal Inactivation

Heat inactivation at 60 °C for 10 min

## Protocol

1. Add the reaction components on ice as shown in the table below (for a 20 µL reaction):

| Component                | Amount      |
|--------------------------|-------------|
| 10X IdeZ Reaction Buffer | 2 µL        |
| Antibody (IgG)           | 10 µg       |
| IdeZ Protease            | 1 µL        |
| ddH <sub>2</sub> O       | Up to 20 µL |

2. Mix the reaction components and centrifuge briefly to collect the solution at the bottom of the tube.

3. Incubate at 37 °C for 30-60 min.

4. Inactivate the enzyme by heating at 60 °C for 10 min.

Analyze the digested products by denaturing or non-denaturing PAGE, stain with Coomassie Blue, and photograph to evaluate the cleavage results.

## Note

1. It is recommended to keep IdeZ Protease on ice or in an ice bath during use and to store it at -20 °C immediately after use.

2. The cleavage efficiency of IdeZ Protease varies considerably among IgG from different species. When cleaving IgG from a specific species, it is recommended to optimize the reaction time and enzyme amount accordingly. Among commonly used species, IdeZ Protease cleaves human and rabbit IgG with high efficiency, whereas its efficiency for rat IgG and mouse IgG2a is relatively low. In such cases, increasing the amount of IdeZ Protease or extending the incubation time is recommended to achieve complete digestion of the IgG antibody.