

# Mass Spec Kit for IgG: Instructions For Use

Catalog no. QA1-1Z2-010

## **Product Description**

When performing mass spectrometry of large proteins, such as IgG molecules, there is often a need to remove the glycans because of poor quality of the data otherwise obtained. Additionally, fragmentation of IgG allows for more detailed analysis of individual components of IgG.

Mass Spec Kit contains both FabRICATOR® and IgGZERO™ enzymes for performing both deglycosylation and fragmentation of 1mg IgG in an hour. Mass spectrometry of samples can then be run directly without further purification.

IgGZERO™ is an endoglycosidase with a very high specificity for IgG molecules of all species and subclasses. IgGZERO™ has a mass of 110 kDa.

FabRICATOR® is an enzyme used for preparation of F(ab')<sub>2</sub>. FabRICATOR® is a digestive enzyme that cleaves IgG only at one specific site below the hinge region resulting in F(ab')<sub>2</sub> and Fc-fragments.

Please note: FabRICATOR® cleaves all subclasses of human, monkey, rabbit and sheep IgG but only subclass IgG3 of mouse IgG.

Since FabRICATOR® only cleaves at one specific site below the hinge region, there is no risk of getting fragments other than Fc and F(ab')<sub>2</sub> even if the incubation time is increased for several hours.

## **Content and storage**

IgGZERO™ is supplied as lyophilized powder from 10mM sodium phosphate, 150mM NaCl, pH 7.4, with no preservatives added.

IgGZERO™ is shipped on ice and should be stored at -20°C upon arrival.

After reconstitution IgGZERO™ is stable for 1 month at +4-8°C.

IgGZERO™ is for in vitro research use only. Not for use in therapeutic or diagnostic procedures.

FabRICATOR® is supplied as lyophilized powder from 50 mM sodium phosphate, 150 mM NaCl, pH 6.6, with no preservatives added.

Contains sufficient material to produce F(ab')<sub>2</sub> fragments from up to 1mg IgG.

FabRICATOR® is shipped on ice. It should be stored at -20°C upon arrival.

After reconstitution FabRICATOR® is stable for 1 month at +4-8°C.

FabRICATOR® is for in vitro research use only. Not for use in therapeutic or diagnostic procedures.

## **Unit Definition**

**IgGZERO™:** One unit cleaves ≥ 95% of 1µg IgG when incubated in 0.01 M sodium phosphate, 0.15M NaCl, pH 7.4 at 37°C for 30min.

**FabRICATOR®:** One unit cleaves ≥ 95% of 1µg human IgG when incubated in 50mM sodium phosphate pH 6.6, 150mM NaCl at 37°C for 30min.

## **Quality Control**

IgGZERO™ and FabRICATOR® are tested to ensure lot-to-lot consistency.

IgGZERO™ and FabRICATOR® are tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates and fluid thioglycolate medium.

## **Additional reagents required but not included:**

**Cleavage buffer 1:** 10mM sodium phosphate, 150mM NaCl, pH 7.4

**Cleavage buffer 2:** 50mM sodium phosphate, 150mM NaCl, pH 6.6.

### Procedure

Reconstitute IgGZERO™ (**A0-IZ1-010, 1000u**) in **50µL** double distilled H<sub>2</sub>O and FabRICATOR® (**A0-FR1-010, 1000u**) in **50µL** double distilled H<sub>2</sub>O. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).

The following protocol is for deglycosylation and cleavage of 1mg IgG. Scale to your sample.

1. Add 1000u (50µl) IgGZERO™ to 1mg IgG.
2. Add 450µL cleavage buffer 1.
3. Incubate 30min at 37°C.
4. Add 1000u (50µL) FabRICATOR®.
5. Add 450µL Cleavage buffer 2.
6. Incubate 30min at 37°C.
7. Finished.

### Product References

Maria Allhorn, Arne Olsén and Mattias Collin: *EndoS from Streptococcus pyogenes is hydrolyzed by the cysteine proteinase SpeB and requires glutamic acid 235 and tryptophans for IgG glycan-hydrolyzing activity.* BioMed Central, January 8, 2008.

Mary H. Ryana, Diane Petrone, Jennifer F. Nemetha, Evan Barnathan, Lars Björck, Robert E. Jordan: *Proteolysis of purified IgGs by human and bacterial enzymes in vitro and the detection of specific proteolytic fragments of endogenous IgG in rheumatoid synovial fluid,* Molecular Immunology, October 2007.

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